The impact of seasonal variations of New Zealand raw milk on the heat stability of skim milk

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Abstract of a thesis submitted in partial fulfilment of the requirements for the Degree of Master of Food Science.

The impact of seasonal variations of New Zealand raw milk on the heat stability of skim milk

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Abstract

Seasonal changes in milk composition is well documented in several countries. The concentration of many constituents and the physicochemical properties differ throughout the year to different extents. In New Zealand (NZ), the milk composition changes during a milking season are linked to the weather cycle. To the best of our knowledge, how seasonal variations of raw milk constituents affect heat stability of skim milk in NZ has not been established. The objectives of this research are to investigate how the season changes affect fresh raw milk components in NZ dairy farms in Canterbury and its impact on the heat stability of skim milk. Milk used in this research was collected fortnightly from the Lincoln University research farms in Canterbury, NZ. During the research period fresh whole milk (FWM) and fresh skim milk (FSM) were used. During the study period, FWM and FSM were measured for the general composition (GC), pH, free calcium ion concentration (Ca^{++}), particle size distribution (PSD), sedimentation rate(DS%), ethanol stability (ES%), total phospholipids (TPL), buffering capacity (BC), and composition of fatty acids (FA), minerals, and proteins. Fresh skim milk was obtained by centrifuging FWM at a rate of 3000 x g for 30 min and it was used to investigate the milk heat stability by eliminating the impact of milk fat globules and their associated materials. Di-sodium hydrogen phosphate (DSHP), tri-sodium citrate (TSC), sodium dihydrogen phosphate (SDHP) and di-sodium hydrogen phosphate (DSHP) were added as stabilizing salts to reduce heat-induced sedimentation. One set of FSM was homogenized at 11,000 rpm for 10 min and heated to 85^0C another set of FSM was heated to the same temperature and then homogenized.

This study showed seasonal variation in milk protein and fat concentrations, pH and BC, TPL, total whey protein and a-casein, Ca^{++}, FA, minerals, surface area mean [D (3, 2)] and volume weighted mean [D (4, 3)], ES%, and DS%. No seasonal variation was evident in total milk solids (TS; %). In addition, the average mineral (calcium, potassium, magnesium, sodium, phosphorus, zinc, and

sulphur) concentrations varied significantly ($p<0.05$) between seasons ($p<0.05$). Stabilizing salts DSHP (salt 1; S1), TSC (salt 2; S2) in the study), and a mixture of 2 parts of SDHP and 1part of DSHP (salt 3; S3) when added to milk, improved the heat stability. The addition of stabilizing salts to milk increased pH, decreased Ca^{++} concentration resulting in a lower sedimentation rate of milk components thus may help in extending the shelf-life.

Keywords: Fresh skim milk, fresh whole milk, heat stability, seasonal variation.

Abbreviations

AA	Amino acids
BSA	Bovine Serum Albumin
CC	Correlation Coefficient
DSHP	Di-sodium hydrogen phosphate
FSM	Fresh skim milk
FWM	Fresh whole milk (or raw milk)
HCl	Hydrochloric acid
HTST	High-Temperature short-time heating
IgG	Immunoglobulin
LPC	Lysophosphatidylcholine
LPE	Lysophosphatidylethanolammine
PC	Phosphatidylcholine
PI	Phosphatidylinositol
PLs	Phospholipids
PS	Phosphatidylserine
PSM	Processed Skimmed Milk
RA	Rumenic acid
SDHP	Sodium dihydrogen phosphate
SDS	Sodium dodecyl sulphate
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
TVA	Trans vaccenic acid
TSC	Tri-sodium citrate
UHT	Ultra High Temperature
LA	linoleic
α-La	α-Lactalbumin
β-Lg	β-Lactoglobulin

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Chapter 1

Introduction

1.1 Background

Milk is one of the oldest foods known to human and widely consumed around the world, with an average composition of water ~ 87.4%, total solids ~12.6%, fat ~3.9%, lactose ~4.8%, proteins ~3.4% (casein ~2.6%, β-lactoglobulin (β-Lg) ~0.32%, α-lactalbumin (α-La) ~0.12%), minerals ~0.7% (figure 1), and minute quantities of other miscellaneous components (Bylund, 1995). The milk composition varies depending on geographic location, the stage of lactation (SOL), breed, animal species, milking system, age, size of the cow, environment, climate, temperature, dietary composition and season (Bansal, Habib, Rebmann, & Chen., 2009; DairyCo., 2013). Studies have shown a negative correlation between environmental temperature and the amount of milk fat and protein, and when the temperature increases the solid fat tends to decline (Lacroix, Verret, & Paquin, 1996; Ng-Kwai-Hang, Hayes, Moxley, & Monardes, 1984). As reported by Ng-Kwai-Hang et al. (1984) and Lacroix et al. (1996) the percentage of fat, protein, casein and all the fraction of nitrogen have been affected by the seasonal variations.

In New Zealand (NZ), the milk composition changes during a milking season are linked to the weather cycle (Bansal et al., 2009). To the best of my knowledge, there is no information on how seasonal variations of raw milk constituents affect heat stability of skim milk in Canterbury, New Zealand (NZ). This research investigates how seasonal changes affect fresh raw milk components in NZ dairy farms in Canterbury and their impact on the heat stability of skim milk. Milk used in this research will be collected fortnightly from the Lincoln University research farms in Canterbury, NZ. Fresh whole milk (FWM) and fresh skim milk (FSM) were investigated for its general composition, pH, Ca^{++} activity, particle size distribution (PSD), sedimentation; ethanol stability (ES), while fatty acid (FA) composition, mineral composition and buffering capacity (BC) was measured from FWM only. In addition, the total phospholipid content and protein composition (SDS-PAGE) was measured for FSM only. Skim milk was obtained from fresh raw milk using centrifugation and was used to investigate milk heat stability by eliminating the impact of milk fat globules and their associated materials. Di-sodium hydrogen phosphate (DSHP), tri-sodium citrate (TSC) and Sodium dihydrogen phosphate (SDHP) and di-sodium hydrogen phosphate (DSHP) as a mixture in a ratio of (2:1) will be added as stabilizing salts respectively to reduce heat-induced sedimentation in skimmed milk. Adding supporting salts might be a solution to overcome the impact of seasonality on heat stability of skim milk in case seasonality is indeed a critical factor (Sweetsur & Muir, 1980).

1.2- Aim and objectives

This research project was designed to improve our understanding on the

(i) Seasonal effects on composition and physicochemical properties of raw milks derived from NZ farms (Canterbury).

(ii) Impacts of seasonal variations on the heat stability of fresh skimmed milk.

1.3- Research questions (Questions & gaps)

Based on the introductory information above, it was apparent that the impact of seasonal variation of fresh milk in NZ on heat stability is not well known. It was also not clear which determining factors of heat stability are associated with seasonality. To our knowledge, no work has been published on the impact of seasonal variations of raw/skimmed milk over a one-year period on the heat stability of skimmed milk in NZ. The research questions to be addressed are as follows: -

1. Is FA composition of raw milk altered between seasons?
2. Are physicochemical properties of raw/skim milks altered between seasons?
3. Which season or month results more heat stable skim milk?
4. What are the possible factors that influence the stability of heat-treated skim milk based on sedimentation rate and colour change rate?
5. What was the impact of high shear (before or after heat treatment) on skimmed milk heat stability?
6. Would the addition of stabilising salts mitigate or eliminate negative effects of seasonal variations on heat-treated skim milk?
6. Is ethanol stability of FWM a reliable indicator of heat stability of FSM in different seasons?
7. Is free Ca^{++} concentration of fresh milk a critical factor in regulation of stability of heat-treated skim milk?

1.4- Research approach

During the research, raw milk was collected fortnightly from the Lincoln University dairy farms in Lincoln, Canterbury, NZ. All experiments and bench scale treatments were conducted at Lincoln University (LU) and/or Plant and Food Research (PFR) laboratories.

Fresh whole milk (FWM) and fresh skim milk (FSM) were measured for their general composition (FSM for fat content only), pH, Ca^{++} concentration, sedimentation and particle size distribution (PSD). Total phospholipid content, fatty acid (FA) composition, mineral compositions, buffering capacity and ethanol stability were measured in FWM, and protein composition analyzed in FSM only. FSM was heat treated in a water bath (85° C for 5 min) and high shear-treated (high shear was applied in same aliquots either before or after heat treatment) with addition of stabilizer salts. PSM was assessed for pH, Ca^{++} activity, sediment rate, colour and PSD (for casein micelles) on day 1 (1 day

after processing) and day 30 (30 days after processing).

Due to the time constraints of a one-year MSc program, FWM samples were collected, treated and analysed twice each month for approximately 9-months (covering 3-4 seasons within a year) leaving three months for thesis writing.

1.5- Hypothesis

Fresh milk (FWM and FSM) composition is of major importance for milk processing. Milk composition affects the physicochemical properties of milk during heat treatment. During the four seasons (9-month only for processed milk, 12 months for fresh whole milk) of the study, both constituents and the physicochemical properties of FWM and FSM may vary between seasons or even between months. Such changes might impact on heat stability of FSM. Heat stability of FSM may be manipulated by the addition of stabilizing salts. A significant correlation would be identified between physicochemical properties of FWM and heat stability of FSM. Principal component analysis (PCA) was able to discriminate the fresh milk sourced from different seasons based on composition, physicochemical properties, and heat stability.

Chapter 2

Literature Review

2.1-Milk composition

Milk is defined in many ways. Chemically, it is defined as a complex fluid in which more than 100 separate chemical compounds have been found. It is also one of the oldest foods known to human and widely consumed around the world, with average composition of water ~ 87.5%, total solids ~13.0%, fat ~3.9%, lactose ~4.8%, proteins ~3.4% (casein ~2.6%, β-lactoglobulin (β-Lg) ~0.32%, α-lactalbumin (α-La) ~0.12%), minerals ~0.8% as shown in figure 1, (Bylund, 1995). While from a physiological viewpoint, milk is the discharge from a healthy mammary gland of the females of all mammals, which is shaped for some time following parturition to nourish the young of a species during the early period of development. Milk for human consumption is obtained from some domesticated animals including sheep, goat, buffalo, and cow, whose milk is by far the most consumed by a human. Bovine milk is the most and main type of milk consumed by a human as it is rich source of essential nutrients such as micronutrients, include calcium, phosphorus, vitamins like B and D, high-quality protein such as casein protein, also fatty acid composition (Frelich et al., 2012). Throughout this report, the term milk refers to bovine milk. The composition of fresh whole milk is not completely constant. It varies depending on geographic location, the stage of lactation (SOL), breed, the species, milking system, age, the size of the cow, the environment, the climate, the temperature, dietary composition and season (Bansal & Chen, 2006).

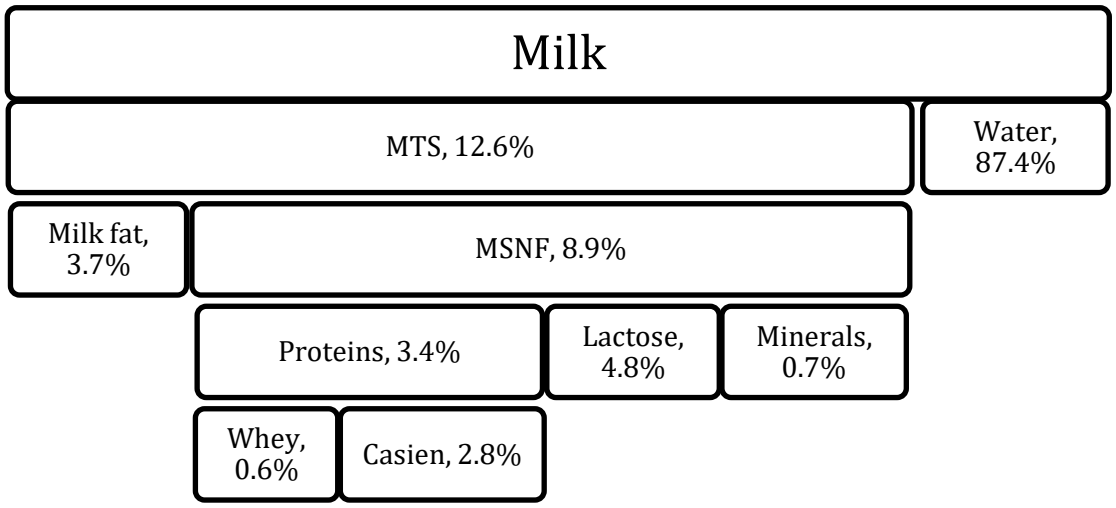

Figure 1: Typical gross composition of milk

Environmental factors, cow nutrition and seasons of the year have a substantial impact on milk components and properties. Milk properties such as taste, colour, fat content and fat type vary with the season (Nateghi, Yousefi, Zamani, Gholamian, & Mohammadzadeh, 2014). In terms of physical chemistry, milk is a dense, whitish fluid of multi-disperse phases.

2.2-Constituents of milk

Milk is composed of water, milkfat and milk non-solid fats as mentioned earlier (Bylun., 1995).

2.2.1-Milk Lipids

Milk lipids in bovine are similar to milk lipids of other species, and are mainly present in globules as an oil-in-water emulsion (Månsson., 2008). Milk fat consists of many triacylglycerols (about 98% of total fat) located inside fat globules (Liu, Wang, Cocks, & Rochfort, 2017). Due to the large number of triacylglycerols in milk fat, triacylglycerol has a significant impact on milk fat properties such as hydrophobicity, density, and melting characteristics. Bovine milk fat contains approximately 400 different fatty acids (Månsson., 2008) with a variety of structures, which makes it the most complex naturally occurring fat (Månsson., 2008). Fats are present in the form of spherical droplets with a diameter of 0.2 to 15.0 µm, with the majority of the fat found in globules diameter of 1.0 to 8.0 µm known as milk fat globules membrane (MFGM). MFGM consists of 70% proteins and approximately 30% lipids, mainly composed of polar lipids and membrane-bound associated with proteins (Mather, 2000).

Table 1: Main classes of lipids in milk %(W/W), adapted from Walstra and Jenness (1984)

Main classes of lipids in milk	
Lipid class	Amount (%, w/w)
Triacylglycerols	98.3
Diacylglycerols	0.3
Monoacylglycerols	0.03
Free fatty acids	0.1
Phospholipid	0.8
Sterols	0.3

Bovine lipids also consist of diacylglycerol (about 2% of the lipid fraction), monoacylglycerol (0.03), free fatty acid (about 0.1), phospholipids (about 1%), sterols (0.3) and cholesterol (less than 0.5%) (Jensen, 2002), it also consists traces of fat-soluble vitamins, β carotene, and fat-soluble flavouring compounds (table 1). Bovine milk lipids (fatty acid and fat) can vary from 3 to 6% based on the cow breed, diet, seasons, and stage of lactation, size of the cows, environment, climate and temperature (Bansal & Chen., 2006). Milk lipids are present in the form of an oil-in-water emulsion droplet; the emulsion droplet is named as milk fat globule (MFG) (Zheng, Jiménez-Flores, & Everett, 2013).

2.2.1.1. Fatty acid

Milk fats are viewed as the most complex fats of all-natural fats, due to a large number of fatty acids (400 different fatty acids) with a different structures (Carrol et al., 2006; Jensen, 2002). About 15 of the 400 fatty acids are present at a rate of 1% or higher, while the remaining exist as traces. Milk fatty acids are derived almost from two sources. These sources are (i) the plasma lipids (account 55% FA) originating from the feed, and, (ii) the microbial activity in the mammary gland of the cow which

generates the even number of carbon 4:0-16:0, and accounts for ~45% of total FAs in milk fat (Parodi, Apr 2004). C4:00 to C14:00 and some of C16:00 FAs are made in the mammary gland of the animal, while C18:00 and some of the C16:00 FAs come from the animal's diet. Long chain FAs account for 40% - 60% of milk FAs, predominately C18 (MacGibbon & Taylor, 2006).

Milk fats contain saturated fatty acids (SFA) and unsaturated fatty acids (USFA) mainly, monounsaturated (MUSFA) and polyunsaturated fatty acids (PUSFA). Saturated fatty acids are milk fatty acids with un-branched hydrocarbons chain, which vary in its length from 4 to 18 carbon atoms. These fatty acids account for approximately 70% - 75% by weight of the total FAs present in milk. Palmitic acid (16:0) accounts for approximately 25% to 30% by weight of the total saturated FAs in milk fats, while Myristic acid (14:0) and stearic acid (18:0) make up 10% and 13% by weight of the total SFAs in milk fats, respectively (MacGibbon & Taylor, 2006) . Short-chain fatty acids (C4:0–C10:0) account for up to 10% of the SFA by weight of the total FAs present in milk (MacGibbon & Taylor, 2006).

Unsaturated FAs are those with one or more than one double bond. Unsaturated FAs in milk consist of MUSFA or PUSFA. Mono-unsaturated fatty acids are FAs with one double bond in the fatty acid chain, while all other carbons are single bonded. These FAs account 18% to 24% of the total FAs in milk fats, with oleic acid (18:1) accounting for 15% to21% by weight of the total FAs(MacGibbon & Taylor, 2006) . Poly-unsaturated fatty acids constitute about 2.3% by weight of the total FAs, linoleic acid (18:2) and α-linolenic acid (18:3) are the main. Trans FAs are those with one or more trans-double bonds and accounts about 2.7% of the FAs in milk

Conjugated linoleic acids (CLA) refers to a mixture of positional and geometric isomer of linoleic acid found mostly in the meat and dairy products derived from ruminants. Milk and milk products are the richest source of CLA that are both accessible and acceptable to most consumers. Milk fat can contain over 20 different isomers of CLA. The most active isomer in CLA group is cis-9, trans-11 CLA. CLA content of milk is synthesised during the biohydrogenation of FA rumen, or in tissues by Δ-9 desaturase enzyme activity (Mosley, Powell, Riley, & Jenkins, 2002). Dairy and dairy products have gained much attention mainly due to health benefits related with conjugated linoleic acid (CLA) isomers, mainly cis-9, trans-11 CLA isomer. The trans-11 CLA isomer also named rumenic acid (RA) that accounting for 75–90% of the total CLA content in milk fat is the most abundant and most biologically active natural isomer of CLA in dairy and dairy products (SOJÁK, 2010).

Table 2: Major fatty acid composition of cows in %(W/W), adapted from (Creamer & MacGibbon, 1996).

Lipid Numbers	Common Name	%(W/W)

C4:0	Butyric acid	3.9
C6:0	Caproic acid	2.5
C8:0	Caprylic acid	1.5
C10:0	Capric acid	3.2
C12:0	Lauric acid	3.6
C14:0	Myristic acid	11.1
C14:1	Myristoleic acid	0.8
C15:0	Pentadecylic acid	1.2
C16:0	Palmitic acid	27.9
C16:1	Palmitoleic acid	1.5
C18:0	Stearic acid	12.2
C18:1-cis	Oleic acid	17.2
C18:1-trans	Vaccenic acid	3.9
C18:2	Linoleic acid	1.4
C18:2conj	CLA	1.1
C18:3	α-Linolenic acid	1
	Minor fatty acids	6

2.2.1.2 Phospholipids:

Phospholipids (PLs), which accounts 0.8% of total milk Lipids, are the essential elements of natural membranes. PLs belongs to the class of polar lipids, which are fundamental in milk for the emulsification of fat in water. PLs and proteins are the main constituents of the milk fat globule membrane (MFGM), which gives MFGM an amphiphilic property that plays a significant role in the milk. This feature, which affects their role, behaviour, and function, is due to the presence of both a hydrophobic tail and a hydrophilic head (Singh, 2006) . Together with PLs, MFGM includes, (glyco)proteins, glycolipids, total and partial glycerides, free FAs and cholesterol (Singh, 2006). The PLs are originated in the apical plasma membrane of the mammary gland secretory cell as most of the MFGM (Dewettinck et al., 2008).

Glycerophospholipids, which are formed by glycerol, phosphoric acid, FAs, a hydroxy compound and sphingolipids that contains a long chain base, and sphingolipids are quantitatively the most important PLs in milk (Beare-Rogers, Dieffenbacher, & Holm, 2001). Both glycerophospholipids and sphingolipids represent about 0.5%–1% of milk fat and about 60%–70% of milk PL, and they are located in the external bilayer membrane of the MFGM (Gallier, Gragson, Cabral, Jimenez-Flores, & Everett, 2010b).

Lysophosphatidylethanolammine (LPE), lysophosphatidylcholine (LPC) and plasmalogen are among the minor PLs detected in milk (Gallier, Gragson, Cabral, Jimenez-Flores, & Everett, 2010a; Garcia et al., 2012; Hay & Morrison, 1971).

2.2.2-Milk proteins

Proteins are polymers of amino acid molecules connected by peptide bonds. There are more than 200 types of protein found in milk, but only a few groups of the proteins are present in large quantities. Milk protein yield is mainly dependent on milk yield and the concentration of protein (Teepker & Swalve, 1988). Bovine milk proteins make 2.5-3.7 % (W/V) of the milk. It consists of about 20% whey proteins with major components α-lactalbumin (α-LA), β-lactoglobulin (β-LG) and 80% caseins, divided into major subclasses α- (αS1- and αS2-), β- casein(-CN), and κ-casein (-CN), which are arranged in micelles (Rodriquez, Mekonnen, Wilcox, Martin, & Krienke, 1985; Swaisgood, 1982). The variations in milk proteins are mostly caused by geographic location, the stage of lactation, breed, species, milking system, age, size of the cows, environment, climate, temperature, dietary composition and seasons (Bansal & Chen, 2006) . Casein protein (CN) and whey protein are the two-major milk protein (Figure 1) that generally define the chemical composition and physical properties of milk. Neither the casein nor the whey protein fractions are homogeneous in their composition. The major milk proteins, the caseins, ß-lactoglobulin, and α-lactalbumin, are synthesised in the mammary gland, while the immunoglobulin and serum albumin are absorbed from the blood. About 60% of the amino acids used to build the proteins are obtained from the cow's diet. Casein protein (CN), whey protein, milk fat globules membrane (MFGM) proteins and enzymes are the naturally occurring proteins (Farkye & Shah, 2014).

Table 3: Protein composition in g/L and molecular weight in KDa of bovine milk. It is modified according to (Dalgleish, 1993; Farrell Jr et al., 2004).

Protein	MW (KDa)	Amount in milk (g/L)	Protein (%)
Total casein		26	79.5
αS1-Casein	23.164	10	30.6
αS2-Casein	25.388	2.6	8
β-Casein	23.983	9.3	28.4
κ-Casein	19.038	3.3	10.1
Total whey		6.3	19.3
β-Lactoglobulin	18.277	3.2	9.8
α-Lactalbumin	14.175	1.2	3.7
Bovine Serum Albumin	66.463	0.4	1.2
Immunoglobulin	103-105	0.7	2.1

2.2.2.1-Caseins

Caseins which makes 80% of bovine milk protein, are phosphoproteins that precipitate from milk at

pH4.6 and temperature of 30℃ (Farkye & Shah, 2014). The caseins are unique phosphoproteins that are in suspension in milk colloidal particles. 95% of the caseins are found in a form of a spherical shape colloidal particles diameter of 100–200 nm, known as caseins micelles (Farkye & Shah, 2014). Micelles has porous structures that allow the water phase to move freely in and out. The caseins are divided into four major components, α-S1, α-S2 and ß and κ-casein which are generally distributed in the proportions 40 %, 10 %, 40 %, 10 %, respectively, of the total casein (Dalgleish, 1990), held together by Ca phosphate nanoclusters, with the κ-caseins protecting it from on the inside, surrounded by a layer of casein which helps to stabilize the micelle in solution (Holt, Carver, Ecroyd, & Thorn, 2013). Caseins do not have clear secondary or tertiary structures (Holt et al., 2013).

2.2.2.2-Whey proteins

Whey proteins are the second largest group of proteins in milk, which are soluble in solution at pH 4.6 and temperature of 20 °C (Farrell Jr et al., 2004). Why proteins make 20% of total bovine milk protein and are found in solution form. Whey protein mainly encompass of four major proteins that represent 90 % of total whey proteins. They are β-Lactoglobulin (β-Lg), α-Lactalbumin (α-La), Bovine serum albumin (BSA) and Immunoglobulins (IgG). Proteins such as lactoperoxidase, serum transferrin, enzymes and milk fat globular membrane protein make 10% of the remaining total bovine whey protein (Fox & Kelly, 2006). Whey can be separated from the casein proteins during coagulation processes of the casein. The whey proteins are highly structured globular proteins, with stable secondary and tertiary structures. This Structure are maintained by major forces of disulphide bonds, hydrophobic interactions, hydrogen bonding, ion-pair interactions and van der Waal's interactions(Singh & Havea, 2003). As the result of the large proportion of hydrophilic residues on the surface of the globular structure and the large amount of disulphide bonds, whey proteins are highly soluble in the milk over a broad range of pH (Dissanayake & Vasiljevic, 2009).

2.2.2.2.1 β-Lactoglobulin (β-Lg)

β-Lactoglobulin (β-Lg) is a small soluble protein that exists as a dimer of subunit molecular weight 18350Da. β-Lg is the major whey protein in bovine milk; it makes 50% of the total whey protein and 12% of total protein content. The β-Lg monomer contains five cysteine residues, of which four form disulphide bonds. There are several genetic variants of β-Lg, with the A and B most common. Both A and B variants consists of 162 amino acid residues per monomer, with one free cysteine and two disulphide bridges (Kontopidis, Holt, & Sawyer, 2004). Both A and B variants differ by two amino acids, with variant A has an aspartic acid residue at position 64 and a valine residue at position 118, while variant B has glycine and alanine in these positions, respectively. These variants contain five cysteine (Cys) residues, located at positions 66, 106, 119, 121, and 160. The cysteines form two disulfide bonds, between Cys66 and Cys160, and between Cys106 and Cys119, while Cys121 is a free

thiol that lies buried in the center of β-Lg structure (Papiz et al., 1986). β-Lg belongs to the protein family of lipocalins. Lipocalins are large group of small extracellular proteins, which transport small hydrophobic molecules such as steroids, bilins, retinoids (vitamin A), and lipids. β-Lg is a highly structured β-pleated protein sheet, formed by nine β-strands and one α-helix (Kontopidis et al., 2004). Beyond the nutritional contribution of the individual components of β-Lg, the biological functions of the complex protein are still hypothetical. The assumed roles are (i) increase of FA absorption(Perez et al., 1992)(ii) modification of the kinetics of the enzymatic hydrolysis of the protein (Puyol, Perez, Mata, Ena, & Calvo, 1993)(iii) protection of sensitive ligands against oxidation (Futterman S & J., 1972), and (iv) modification of the bio-accessibility of the ligands (Puyol, Dolores Perez, Sanchez, Ena, & Calvo, 1995).

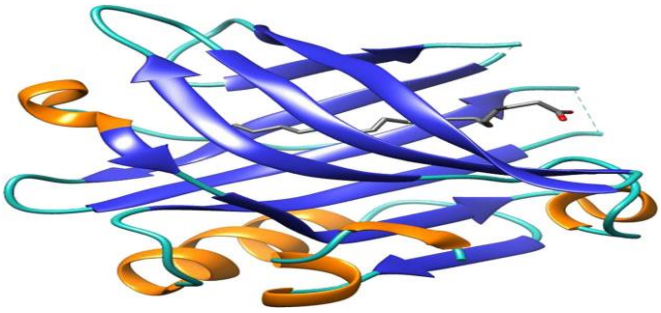

Figure 2: A schematic view of main-chain β-Lg

2.2.2.2.2 α-Lactalbumin

Alpha-Lactalbumin (α-La) is a small (Mw 14kDa), acidic and Ca++ binding protein. α-La is the second major whey protein in milk and makes 25 % of the total whey protein in milk, with 123 amino acids residue. The α-LA has eight cysteine residues, forming four disulfide bonds.

The α-La is important for several points. Some of these points are:- (i) α-La is one of the two components of lactose synthase, which regulates the production of lactose in the milk of almost all mammalian (Hill & Brew, 1975). The concentration of lactose in milk is directly related to the concentration of α-La (Caffin, Poutrel, & Rainard, 1985). (ii) α-La strongly binds calcium and zinc ions. Due to its single strong Ca^{++} binding site, α-La is frequently used as a simple model Ca^{++} binding protein (Hiraoka, Segawa, Kuwajima, Sugai, & Murai, 1980). The strong Ca^{++} binding site of α-La is important for the its stability during heating, as calcium increases the stability of α-La. α-La consists of two domains which are connected by a calcium-binding loop. These domains are:-(i)a large α - helical domain, which is composed of three major α- helices (residues 5-11, 23- 24, and 86-98) and two short 310 helices (residues 18-20, and 115-118), and(ii) a small β-sheet domain, which is composed of a series of loops, a small three-stranded antiparallel β-pleated sheet (residues 41-44, 47-50, and 55-56) and a short 310 helix as in figure 3 (three residues per turn and an intrachain hydrogen bond loop containing 10 atoms; residues 77-80) (Permyakov & Berliner, 2000).

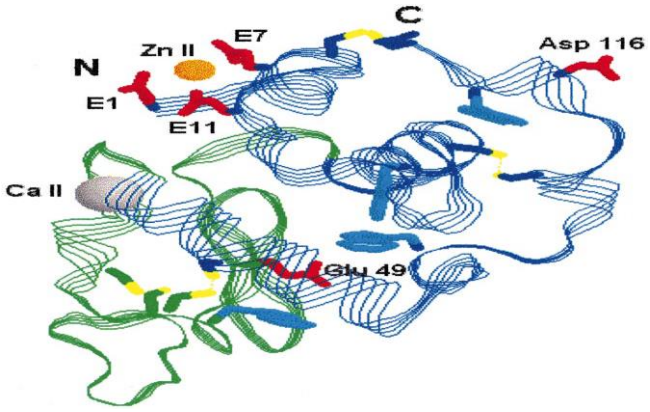

Figure 3: X-ray alpha-Lactalbumin (α-LA) structure from native buffalo protein.

2.2.3-Milk minerals

Minerals which represent about 8-9 g/L, occurs in different chemical forms such as inorganic ions and salts or as parts of proteins, nucleic acids, fats and carbohydrates (Zamberlin, Antunac, Havranek, & Samaržija, 2012). The minerals content of milk varies with stage of lactation, feed, genetic variance etc. Calcium (Ca), phosphorous (P), Magnesium (Mg), Sodium (Na), Potassium (K) and chloride are the major minerals found in milk (Carter & Chesson, 2017). In milk, approximately 67% of the calcium, 35% of the magnesium, and 44% of the phosphate exist as salts, bound within the casein micelle and the remaining are found as soluble in the serum phase. The fact that Ca and phosphate are associated as salts bound with the protein does not affect the nutritional availability of either Ca or phosphate. The average concentration, standard deviation and range of these minerals calculated from the literature (Gaucheron, Le Graët, Piot, & Boyaval., 1996; Summer et al., 2009) as in table 4. In milk, ions play an important role in the structure and stability of casein micelles (Gaucheron et al., 1996; Holt., 2002).

Table 4: Major minerals in cow milk in mg/L adapted by (Gaucheron et al., 1996)

Mineral	Mean, mg/L	SD, mg/L	Range, mg/L
Calcium	1194	39.2	1,120-1,235
Phosphorus	1112	406	825-1,995
Magnesium	117	8.54	100-125
Sodium	531	83.7	446-669
Potassium	1550	136	1,360-1,769

Cow milk is an essential source of Ca, with an average concentration of 1194 mg/L (Table 4). Calcium is present relatively in high concentration in the milk of many species mostly found as inorganic ions and salts. Calcium in milk is found in colloidal Ca and soluble Ca^{++}, in which two-thirds of the Ca is in

the colloidal phase [e.g. casein micelles or as Ca^{++}) bound to phosphoserine , while the rest is in a soluble form (Cashman., 2006), which constitutes approximately 10% of the total Ca (Holt & Jenness, 1984). Ca^{++} concentration in bovine skim milk ranges from 2.0 to 2.3mM, which is less than 10% of the total Ca in milk (Christianson, Jenness, & Coulter, 1954). Ca is an important component of milk casein micelles and it plays a role in the stability of the micelles themselves. Its concentration is affected by the number of caseins (Carrol et al., 2006).

Cow milk is a dynamic source of P, with the average concentration of 1,112 mg/L (Table 4). 20% of the total P is present in an organic form bound to molecules such as proteins, organic acids, phospholipids, and nucleotides, mainly in colloidal phase, and an inorganic form. P mostly present as ionic P in a soluble fraction (Gaucheron et al., 1996), while the remaining 80% as in inorganic phosphate. 44% of the total inorganic phosphate is bound to casein micelles as Ca phosphate while 56% in free phosphate ion form (Cashman., 2006).

Bovine skim milk contains magnesium (Mg) with an average content of 117 mg/L (Table 4). 65% of the total Mg in the milk is found in soluble phase as magnesium citrate (40%), magnesium phosphate (7%) and 16% free ions while the remaining as colloidal phase bound to casein micelles. The Mg^{++} in skim milk rage 0.82 mM to 0.85 mM (Christianson et al., 1954).

Cow's milk is an excellent source of K, with an average concentration of 1,550 mg/L (Table 4), mainly found in aqueous phase (Cashman., 2006).

Bovine milk contains low Sodium, with an average of 531 mg/L (Table 4). Sodium is mainly found as free ions, but it can also be found bound to chloride (Zamberlin et al., 2012).

2.3-Physical Properties of Milk

2.3.1-Milk pH

The pH of fresh whole bovine milk at room temperature normally varies within a quite narrow range of 6.6 and 6.8 (Chavez, Negri, Taverna, & Cuatrín., 2004; Tsioulpas, Lewis, & Grandison, 2007; White & Davies., 1958). There are many components in milk which provide a buffering action. The major buffering groups of milk are caseins and phosphate. In a study conducted by Tsioulpas et al. (2007) the pH of fresh whole milk do not change between cow's lactation stages, which oppose to a work done by White and Davies. (1958) found that the pH was low in early lactation, increased significantly in mid-lactation reaching its élite in late lactation.

2.3.2-Milk ethanol stability (ES)

Milk ethanol stability (MES) is defined as the minimum percentage of ethanol (v/v) in an aqueous ethanol solution which causes coagulation when added to an equal volume of milk (Horne & Parker, 1980). MES test is used to determine the susceptibility of bovine milk to coagulation by heat (heat stability). Ethanol at high concentrations causes casein to precipitate from milk. MES is controlled by

the Ca concentration in the milk. The factors which affect the concentration milk Ca are such as pH and ionic strength, therefore affect the stability to alcohol.

MES is therefore often determined as a directory of the stability of the milk to heat processing (Gordon, 1993). MES had been the subject of considerable interest for the following reasons (Chavez et al., 2004).

(i) To achieve a better understanding of the factors that control micellar stability.

(ii) For transferring this knowledge to formulate new dairy products or to extend the shelf life of the existing products (Horne & Muir, 1990).

(iii) It is a cheap, efficient and quick pass-or-fail test to detect milk sourness in many countries.

MES test was found to triggering confusions, were good quality milk was rejected as the result of positive result to the test. Accordingly, lack of reliability of MES at certain seasons was recognised (Chavez et al., 2004). Negri (2002) reported lack of reliability for MES test during autumn and spring in some Argentinean dairy farms and a similar report was published by Donnelly and Horne (1986) during winter in Ireland. Both scholars suggested high salts (Ca, Mg, P, and citrate) balance ratio during late and early lactation as main contribution to this behaviour. The ionic Ca concentration (Donnelly & Horne, 1986; Horne., 1987), ionic strength (Horne., 1987) and the pH of milk (Horne & Parker, 1980) played an important role MES.

2.3.3-Milk buffering capacity (BC)

Buffering capacity (BC) is the ability of a solution to resist change in pH. BC is one of the most important physicochemical characteristics that are used to determine the acidity and alkalinity of milk. The effect of BC depend on several milk small constituents such as inorganic phosphate, citrate, organic acids and milk proteins mainly caseins and whey protein (Salaün, Mietton, & Gaucheron, 2005). The physicochemical characteristics of milk can be affected by natural and/or induced variations in the composition, which can affect milk pH. Changes in milk pH can also result during some technological treatments. A buffering capacity value at each pH can be determined graphically by measuring the slope of the pH tangent. Van Slyke (1922) defined a dB/dpH ratio to calculate buffering capacity in a defined pH range.

2.3.4-Colour

Milk colour ranges from a bluish – white to a golden yellow or yellowish white. The white colour of milk is due to the scattering of imitated light by the vital ultramicroscopic particles, fat globules, colloidal casein micelles, and calcium phosphate, while the yellow colour of the milk is due to the fat-soluble carotene pigment that was found in the green plants (Hui, 1993). Milk whiteness is directly proportional to the size and number of particles in suspension. Homogenization increases the

surface area of fat globules significantly. Hence, homogenized milk is whiter than their unhomogenized.

2.3.5-Dry sedimentation in milk

Dry sedimentation rate in milk (DS%) is a storage stability problem in processed milk (heat treated milk). DS can happen either directly after processing or during storage. If the sediment starts to appear immediately after processing, then the sedimentation would have begun during processing time.

During heat treatment process, milk frequently forms aggregates of denatured protein, fat, lactose, and inorganic salts, that deposit or cluster depending on their size of specific weight, and electric charge (Datta, Elliott, Perkins, & Deeth, 2002) . The rate of DS depends on the quality of raw milk (milk compostions), the type and the severity of heat treatment, homogenising pressure, and the storage temperature (Datta, Elliott, Perkins, & Deeth, 2002) . Heat resistant enzymes (plasmin and proteases) in milk determines the biological quality of the raw milk. During heat treatment of raw milk, some of the heat resistant enzymes survive and causes proteolysis of caseins, which results in the coagulation of the hydrolysed caseins, which will form sediment (Chavan, Chavan, Khedkar, & Jana, 2011).

2.4-Milk seasonality changes

Seasonal changes in milk lactation period are mostly caused by regional climatic conditions. Milk seasonality can be defined as the change in composition, quality, and suitability for processing into a dairy-based product throughout the year. Milk safety and quality depend on the milk composition, which is affected by locality, stage of lactation (SOL), breed and species, milking system, age and size of the cow, environment, climate, temperature, dietary composition, and season (Uallah et al., 2005). The main reasons for seasonal milk changes are SOL and environment. Lactation change in milk composition is defined as the compositional change that take place during the time of milk production from parturition to drying-off time, mainly as the physiological change in the mammary gland in health cow.

In New Zealand (NZ), the milk composition changes during a milking season, and this is linked to the weather cycle (Bansal., Habib., Rebmann., & Ch., 2009). The NZ dairy industry is based around the use of pasture as a means of low-cost feed source, which led to the adoption of seasonal calving to maximise the use of grazing (Auldist, Walsh, & Thomson., 1998). The difference in the season of the year is often related to different food management, grown for cows feed. Since the season of the year affects the food intake, Rajčević, Potočnik, and Levstek (2003) agreed that the changes in milk component are more correlated to feeding than to genetic factor. In summer, days are getting longer and the temperature is rising, the rise in temperature results in heat stress to cows, which decreases

dry matter intake (DMI) in cattle, and that decline 35% in milk production (Cowley, Barber, Houlihan, & Poppi., 2015).

Most cows calved just before spring (cows are on the similar stage of lactation) and dried for 8 to 10 weeks during winter starting from late autumn. This practice results in the irregularity of both milk quantity (winter ceases of milk production) and composition (autumn/winter advanced stage of lactation), which attended milk seasonality in the manufacturing properties of milk (Lucey, 1996). Milk seasonality is defined as the change in composition, quality, and suitability of milk for processing in dairy product throughout the year. The seasonal changes in milk composition and quality are related to different factors. These factors are,

(i) Nutritional and environmental factors, accompanying with availability and quality of pasture, and climate change

(ii) The stage of lactation (SOL), associated physiological changes of the herds, as the result of change in the herds mammary gland and

(iii) Pathological factors (e.g., mastitis) (Kefford, Christian, Sutherland, Mayes, & Grainger, 1995).

Lactational change in milk composition is defined, as the changes in milk composition that take place between parturition and drying-off period. SOL has a markedly effect on milk composition (O'brien & Guinee, 2011) .

The New Zealand farming year is divided into four seasons, depending on the time of the year. (i) Spring (October to December), when the cows produce the most milk, with the concentration of protein and fat higher and lower lactose (GoDairy, (2011)). (ii) Summer (January to March), the milk production declines.

Table 5: The definition of different seasons and size of samples during the seasons

Seasons	Abbreviation	Definition	Sample Size
Spring	SP	October, November, December	6
Summer	SM	January, February, March	5
Autumn	A	April, May, June	5
Winter	W	July, August, September	6

iii) Autumn (April to June), the last few months of milking of the lactation cycle. Autumn is the dry period and extends into winter (to end August). (iv) Winter (July to September) as in Table 5. During these lactation periods, milk constituents vary (DairyCo., 2013)

2.4.1-Seasonal changes in milk composition: -

The effect of seasonal variations on milk composition is, as result of the interactions between physiological, climatic, pathological, and feeding factors (Malacarne et al., 2005). It was reported, there is a negative correlation between environmental temperature and the amount of milk fat and

protein (Ozrenk & Inci., 2008). When temperature is increased the solid fat tends to decrease. It has been noted that the changes in the total solids (TS), protein, fat, casein, lactose, and mineral concentrations are associated to seasonality(Bansal et al., 2009) , and SOL (O'brien & Guinee, 2011). In bovine, the milk yield increases during the first six weeks from parturition and then decline toward the end of the lactation period. In early lactation (EL) milk yield goes up to its peak, with reduction in the level of TS, proteins, casein, and fat, while the yield goes down toward late lactation(LL) period with the increase in TS, proteins, casein, and fat (O'brien & Guinee, 2011). Fat and the protein composition of milk are affected by the time of the year Kefford et al. (1995). It was reported that fat and protein were high in winter, while low in summer (Kefford et al., 1995). The decline in fat content more likely to be due to increase in temperature, as it is obvious, it affects the synthesis of FAs during the hot season (Adeela Yasmin, Huma, Butt, Zahoor, & Yasin, 2012). The same scholars also reported, that the concentration of fat, protein, casein, whey protein, blood serum albumin (BSA), and Na are high in late lactation (LL) period and low in early lactation (EL), while immunoglobulin (IgG) concentration are low in early lactation (EL) and high in late lactation (LL). Another study by the same authors reported that the level of casein, BSA, lactose, K, and the ratios of casein to whey protein, protein to fat were low during summer, while whey protein, fat, total protein, and the immunoglobulin concentration high during winter.

In a study conducted in Australia on the effect of heat stress on protein content, Cowley et al. (2015) reported that protein content was lower in summer. The temperature increase in summer decreases the protein content of milk (Sevi et al., 2001). The decreases the protein content is as the result of heat stress; the cow may be using more amino acids (AA) for energy production to meet additional requirements during heat stress period, which reduce the amount of AA available to produce proteins. AA are the primary building blocks of protein. It was reported that the casein and whey protein proportion are also affected over the lactation period. Alpha-S1 casein as a percentage of the total casein drop down on the first few days of lactation, then increases, then remains constant throughout the remaining lactation period, while ß-casein is low during the initial SOL, then goes up (O'brien & Guinee, 2011).

A study was conducted in Pakistan which investigated the general seasonal effects in fresh milk. The results showed that fat composition is the most sensitive component that changes due to seasonal variation (Millogo, Ouedraogo, Agenauml, & Svennersten-Sjaunja, 2009). As reported by Dobranić, Njari, Samardžija, Mioković, and Resanović (2008), both fat and protein contents are higher in autumn and winter while lower in spring and summer.

In a study conducted in Egypt, do Nascimento Rangel et al. (2011) reported an inverse relationship between milk yield and component percentages. These authors also reported that the milk

production in summer was higher, but the percentage of fat and protein were lower when compared with the production in fall and winter.

2.4.2-Seasonal changes in milk minerals

Minerals content in milk is affected by many factors, such as breed, additive genetic effects, lactation stage, parity, herd, feeding management, health status of the mammary gland and seasons. As lactation period is seasonal, minerals concentration changes across lactation. Calcium, P, and Mg concentrations are high at the beginning of lactation period, then decline rapidly till 6 to 8 weeks then rise afterward (Toffanin, De Marchi, Lopez-Villalobos, & Cassandro, 2015) which challenged to finding by (Haug, Høstmark, & Harstad., 2007; Van Hulzen, Sprong, Van der Meer, & Van Arendonk, 2009; Zamberlin et al., 2012). The Ca^{++} in milk and has a vital importance. Bovine milk was found to contain 2.5 to 3.4 mM/liter Ca^{++} (Tessier & Rose, 1958).

2.4.3-Seasonal change in milk ethanol stability (MES)

Milk ethanol stability (MES) is defined as the minimum concentration of added aqueous ethanol that gives rise to milk coagulation (Horne & Parker, 1980). Many studies has shown seasonal variation in the ethanol stability of milk, autumn and spring recorded lower stability (O'brien & Guinee, 2011). The stability of milk protein to ethanol is affected by the concentration of milk salts and different ionic strength and pH.

2.4.4-Seasonal changes in fatty acid

In a study conducted by Lynch et al. (2005), it was found that diet was responsible for 95% of the variance in milk fat. Milk FA acid composition can be influenced by several factors, many of which are an interaction between stage of lactation, seasonal variation, feeding, genetic variation and other factors. Fats in milk are of the most complexes of all natural lipids, that contains various FAs (Jensen & Clark, 1988), that accounts 66% saturated, 30% monosaturated and 4% polyunsaturated (Posati & Orr, 1976). Seasonal discrepancy on fatty acid composition of milk fat has been broadly assessed. Different studies carried out in Austria, Germany, France, and Switzerland showed that the FA composition of bovine milk varies with seasons (Ferlay, Agabriel, Sibra, Martin, & Chilliard, 2008). Milk fat in summer contained less stearic (18:0) and oleic (18:1) acids and more palmitic acid (16:0) in winter, these changes, in FA composition are ultimately due to dietary effects (Jensen & Clark, 1988). Fresh pasture grasses and a hay-concentrate mixture containing higher proportions of linoleic acid (18:2) which influence on the seasonal differences in milk FA composition (Christie, 1979).The fat percentages of milk are also affected by SOL. Milk fats percentages are highest on parturition, then decline for the next eight weeks, followed by a slow increase until the end of lactation (Linn, 1988). During this time (first days of lactation), milk fat contains lower levels of short-chain FAs, with the increase of palmitic acid (16:0) on the next 15 days (Senft & Klobasa, 1970). The highest butyric

acid (4:0) is during the first month of lactation time, while caproic (6:0) to myristic (14:0) acids all increases during the first 4 to 8 weeks of lactation, remains unchanged till the fifth or sixth month, then decreased toward the end of lactation. Milk from early lactation found to have a high amount of MUSFA, mainly C18:1 than mid-lactation and late lactation (Kefford et al., 1995).

Most scholars agreed that short-chain FAs increase for the first 8 to 10 weeks of lactation, with exception of butyric (4:0), while stearic (18:0) and oleic (18:1) acids decrease and palmitic acid (16:0) remains unchanged. Changes happen after the 10th week of lactation tend to be relatively insignificant (Christie, 1979).

The formation of CLA is naturally from dietary linoleic (LA), alpha-linolenic (ALA) and trans vaccenic acid (trans-11 18:1, TVA) FA. TVA is most active isomer in CLA. As CLA is synthesised in the rumen during biohydrogenation of LA, while TVA is synthesised during biohydrogenation of LA, ALA and oleic acid isomerization, the increasing of forage rich in FA possible will increase the production of CLA in the ruminants. Foraging is likely the best natural approach for increasing CLA content in milk of ruminants (Mosley et al., 2002). The content of cis-9, trans-11 CLA in milk fat originating from pasture feeding of cows has been reported to range from 0.22 to 0.52% (Zegarska, Paszczyk, Rafalowski, & Borejszo, 2006)

2.4.5-Seasonal changes in milk phospholipids

Seasonality has a significant impact on that the phospholipids content of whole milk (Holden, Aceto, Dellamonica, & Calhoun, 1966), with the highest PL, PC, and PE produced during LL, while SP was low during ML (Walker, Wijesundera, Dunshea, & Doyle, 2013) . The higher phospholipid concentration in milk is associated with small fat globules (Lopez et al., 2011) in autumn (late lactation) compared with spring (early lactation) milk (McDowell, 2009).

2.5-Milk processing and processing induced changes

2.5.1-Heat

Regardless of its final use, heat treatment is one of the significant milk processing steps in the dairy industry. Milk is heat treated to limit both bacterial load and enzyme activity and extend the shelf life of the final product. As the result of the heat treatment, heat-induced changes are the main issues, which results in of significant process-related product flaws during processing (Beeby, Hill, & Snow, 1971; Burton., 1978; Harper, 1981). Pasteurisation and sterilisation are the most commonly used heat treatments in the dairy industry. There are different heat treatment methods in the dairy industry, and among these the high-temperature and short time (HTST) is the most common. Raw milk is treated to heat 72-80 ℃ for between 15 to 30s in the HTST method, while in low temperature and long-time (LTLT), milk is heat treated to 63℃ for 30min (Lewis & Deeth, 2008). Lewis and Deeth (2008) reported less chemical change in HTST milk. Ultra-high temperature (UHT) is

a heating process, in which milk is heated to 135-150 °C for 10 to 1 seconds and used to destroy all microorganisms and spores in the milk for achieving prolonged shelf life (up to 9 months) at ambient storage conditions (Fox & Kelly., 2006).

2.5.2-High shearing (Homogenization)

Processing conditions have a direct influence on behavioural features of proteins in a milking system. During the processing, milk is exposed to several processing steps such as pumping, stirring, mixing, ultrafiltration and homogenising, which in turn produce hydrodynamic shear stress on the proteins resulting in destabilisation of native structures leading to denaturation and aggregation (Bekard, Asimakis, Bertolini, & Dunstan, 2011; Chandrapala, Martin, Zisu, Kentish, & Ashokkumar., 2012; Chandrapala, Zisu, Kentish, & Ashokkumar., 2013). In shear studies, usually, two joint flow fields are applied known as extensional flow and simple shear flow. The fluid mechanical shear is commonly measured as a shear rate which is also known as velocity gradient (Thomas & Geer, 2011). Charm and Wong. (1970) studied the change in proteins as the result of Shearing, where catalase and carboxypeptidase (enzymes) were subjected to high shearing, resulting in the breakage of tertiary structure. The shearing rate and exposure time have great importance in terms of loss of activity (Charm & Wong., 1970).

Homogenization has become a regular industrial process, generally practised as a means of stabilising the fat emulsion against gravity separation. Significant shear-induced changes in the milk are caused by the homogenization process (Anderson & Cawston., 1975). Homogenization primarily causes disturbance of fat globules into much smaller one. The effect of high shear homogenization into the emulsion physical results was dependent on the speed applied to the homogeniser pump, with an active over 3600 rpm speed. At a rate over 3600 rpm a change in the volumetric relationship between the droplets take place. Reduction in the more massive droplets population takes place (Silva, Cerize, & Oliveira, 2016). The impact of both shear and temperature on the aggregation behaviour of whey proteins have been studied by (Steventon, Donald, & Gladden., 2005; Taylor & Fryer., 1994; Walkenström, Windhab, & Hermansson, 1998).

2.5.3-Heat stability

The heat treatment of milk during processing operations results in several physicochemical changes in the milk constituents. The ability of milk to withstand high processing temperatures (over 80°C) without visible changes (coagulation or gelation) is known as heat stability. Heat stability is the most critical parameter of milk quality. On the study done to solve coagulation problems between 1900 and 1960, Sommer and Hart (1922) reported that mineral balance was the important factor of milk heat stability. If the milk is too acidic, it has inadequate Ca and Mg while milk with insufficient of phosphate and citrate is too basic. The heat stability of milk that result from mineral imbalance can

be treated by adding some salts. When milk heated, it causes the precipitation of Ca phosphate and lowered (Ca^{++}). The addition of Ca to milk precipitated both phosphate and citrate but increased (Ca^{++}); while addition of phosphate precipitated Ca and decreased (Ca^{++}), and the addition of citrate dissolved colloidal phosphate but decreased (Ca^{++}) (Tessier & Rose, 1958). Adding salt as heat stability stabiliser was criticised by Rogers, Deysher, and Evans (1921), who showed pH and mineral imbalance of milk, not the factors that affect heat stability in condensed milk, while Rose (1961) discovered the importance of pH on heat stability.

During a research in 1970s, on the effects of processing and compositional factors on the pH dependence on the heat coagulation time (HCT) of milk, the study revealed the roles of β-lactoglobulin and κ-casein, milk salts and urea in heat coagulation. Over the past 40 years, an extensive works review on the heat stability of milk has been studied (Fox, 1981; Fox & Morrissey, 1977; O'connell & Fox, 2003; Singh, 1988, 1995; Van Boekel, Nieuwenhuijse, & Walstra, 1989).

Heat stability of milk can be assessed by sealing a milk sample in a glass tube and placed it in a temperature-controlled oil bath, usually at 140℃ until a coagulum can be seen, the time milk starts coagulating is known as the heat coagulation time (HCT). Milk pH is one of the most critical factors that affect the HCT of milk; the stability of milk increases at higher pH value.

Ethanol test, a whitening test, a protein sedimentation test and a viscosity determination are also other means of assessing heat stability of milk (Singh, 2006) . Heat stability is also affected by the concentration of Ca^{++}, salt concentrations, β-lactoglobulin, κ-casein ratio and ratio of different caseins (B. O'brien & Guinee., 2011) . Various studies including O'brien and Guinee (2011) showed that milk heat stability is high during summer and minimum during winter and autumn.

The heat treatment of milk during processing results in several physicochemical changes (gelled and coagulation) in the milk constituent, which can affect the nutritional value and functional characteristics regarding stability and sensory attributes (Fox & Morrissey, 1977). Some of the changes are listed in table 6.

Ethanol stability (ES) was used broadly as a simple indicator of cow's milk freshness, and it is also used as a quick indirect test to evaluate heat stability (Horne, Parker, Donnelly, & Davies, 1986). It is defined as the minimum concentration of ethanol added to milk that gives rise to coagulation in a fresh milk sample (Horne & Parker, 1980).

2.5.4 Heat-induced changes in milk

Regardless of its final use, heat treatment is one of the significant milk processing steps in the dairy industry. Heat-induced changes relates directly to the intensity of the heat during processing. As the result of the heat treatment of milk a significant change arises on the physicochemical composition and properties of milk, this includes the denaturation of whey proteins, the interactions between the

denatured whey proteins and the casein micelles, the alteration of soluble Ca, Mg, and phosphate to the colloidal state (Macej, Jovanovic, Seratlic, & Barac, 2004; Singh & Waungana, 2001).

Table 6: The effect of heat on milk components adopted from Fox and Morrissey. (1977)

Milk composition modified	Changes during heat treatment	Major consequences
Lactose	It decomposes and form of organic acids.	Effect the lactic acid bacteria, reduce pH, caramelisation
Lactose and proteins	Decomposition with the formation of lactulose, heptulose.	Reduction in nutritive value of protein
	A reaction between aldehyde and amino groups producing Maillard reaction	
Whey proteins	The appearance of active SH groups and H_2S, denaturation of whey protein, inactivation of IgG	Cooked flavour, reduction in oxidation-reduction potential, production of lipid antioxidation properties, aggregation and loss cream ability
Whey proteins and casein	Development of ammonia, concentration and an insolubilization of a liquid-air interface, a formation of the complex between K-casein and β-1actoglobuli	Effects flavour, development of "scum" layer on boiling, support in stabilisation to further heat processes; improved body in cultured product
Casein	Dephosphorylation, peptide bond cleavage, loss of glycol-macropeptide from K-casein, accompanied by the modification in the casein micelle structure, formation of Lysino alanine under alkaline condition	Increased sensitivity of calcium precipitation, coagulation of caseins at high temperature
Minerals	Displacement of the equilibrium of Ca/P soluble to Ca/P insoluble salts, modification of the nature of the surface of the micelle	Precipitation of calcium salts and a reduction in pH, delay of rennet coagulation Affects casein micelle stability
Vitamins	Destruction of vitamin C, B (1,6, and 12)	Decrease its nutritive value
Enzymes	Inactivation of enzymes at temperature between 60 to 100°C, reactivation of the enzyme in UHT	Off-flavour and age gelation
Milk fats	Formation of methyl ketones	Causes a coconut flavour

Awareness of heat-induced variations in the milk system has expanded significantly during the past 25 years, filling in details of significant process-related product faults. Still, certain long problems stay partially obvious. These include mechanisms in heat stability and age gelation. The degree of heat-induced changes relates directly to the strength of the heat processes, and these processes can be divided into three categories. These categories are (i) mild, pasteurisation, (ii) medium, ultra-pasteurisation (166 C-15 s); and (iii) severe, sterilisation at 120°C for more than 15min.

The significant heat-induced findings are (i) the interaction between beta-lactoglobulin and kappa-casein (ii) Enzyme reactivation, difference between heat coagulation and heat-induced age gelation, lysine-alanine formation, and additional definition of shifts in ionic equilibrium

2.5.4.1- Effect on the milk proteins

The heat-induced changes in milk are of great practical importance to the dairy industry. Casein protein (CN) and whey protein are the two-major milk protein. The whey proteins are more sensitive to heat than the caseins. Whey proteins denaturation (unfolding) is one of heat-induced changes that takes place when milk is heated to a temperature 65℃ and above. Interaction between beta-lactoglobulin and kappa-casein, enzyme reactivation lysine-alanine formation, in ionic equilibrium and age gelation are some of the heat-induced problem caused by the level of heat intensity during processing which remains unresolved (Burton., 1978; Fox & Morrissey., 1977). The interaction between beta-lactoglobulin and kappa-casein occurs during heat treatments where more than 50% of the whey protein has been denatured (McKenzie., 1971; Sawyer, 1969). As reported by Morr. (1975), this change elaborate multiple interactions, including thiol to disulfide bond formation, hydrogen bonding, hydrophobic interaction, and Ca moderated interactions. Heat-induced coagulation is one of the problems raised during heat processing of milk, its mechanism still has not been outlined but the role of excess Ca ion and shifts in ionic equilibrium are involved (Fox & Morrissey., 1977; Morr., 1975).

When milk is pasteurised (HTST), it will not affect the nutritional and functional properties of the whey proteins. This treatment may cause denaturation of ß-lactoglobulin. Severe heat treatments such as UHT may cause some damage to heat sensitive amino acids and slightly decrease the nutritional content of the milk. The whey protein α-lactalbumin, however, is very heat stable. The denaturation causes a change in the physical structure of proteins, but generally does not affect the amino acid composition and thus the nutritional properties. Amongst the heat-induced changes caused by denaturation of whey proteins are:

(i) Development of cooked flavour

(ii) Development of anti-oxygenic properties

(iii) Impairment of clotting properties

(iv) Imparting of soft curd characteristic to milk

(v) Prevention of age-thicking in evaporated milk

(vi) Improvement in the baking quality for non-fat dry milk in the bakery industry

Caseins is the major milk protein which represents 75-80 % of all milk proteins (Cheaib & Lussi, 2011). It belongs to the group of heat-stable proteins, because it does not coagulate when it is subjected to a high-temperature treatment(140 °C for 15-20) (Jollès, 1979). Its heat resistance is due

to their loose structure. To some extent, both dephosphorylation and hydrolysis of the caseins has been found in heat-treated milk (Belitz, Grosch, & Schieberle, 2004; Farrell et al., 2004; Fox, 1981). Whey protein represents about 18-20% out of total of milk proteins and contains four major proteins: β-lactoglobulin (β-Lg) 50%, α-lactalbumin (α-La) 20%, blood serum protein (BSA) 10% and immunoglobulin (Ig) 10%. Whey proteins has a globular structure, which makes them heat unstable, as the result, whey proteins denaturation (unfolding) takes place at a temperature above 65℃, but it mostly happens at temperatures above 80°C. Whey protein denaturation is a two-step process, protein unfolding followed by aggregation (De Wit & Swinkels, 1980; Mulvihill & Donovan, 1987; Roefs & Kruif, 1994). At the temperature above 65℃, the interactions between the denatured whey proteins and the casein micelles, the conversion of soluble Ca, Mg and phosphate to the colloidal state takes place (Singh & Waungana., 2001) . The level of whey protein denaturation depends on the time, the temperature of treatment, pH of milk, ionic strength (McSwiney, Singh, & Campanella, 1994; Oldfield, Singh, Taylor, & Pearce, 2000; Qi, Brownlow, Holt, & Sellers, 1995) and the level of β-Lg denaturation, since it represents about 50% of all whey protein (Morr., 1985). Due to the differences in the structure and the strength of intermolecular bonds of whey proteins, whey proteins are different in the extent of heat stability. Immunoglobulin's and BSA are the least stable whey proteins, while β-Lg is intermediate and α-La is the most resistant protein to heat denaturation (PP> α-La> β-Lg>BSA> Ig)(Anema., 2014; Corredig & Dalgleish., 1996).

During the heat treatment, milk proteins may interact resulting chemical complex known as co-aggregates of milk proteins. The co-aggregation of proteins is as the result of the interaction between α-La and β-Lg, α-La, and κ-casein, as well as the complex between β-Lg and κ-casein, develops. It also affects the chemical composition during processing (Singh & Waungana., 2001). The formation of milk protein co-aggregation can be explained in two mechanisms. (i) The first mechanism is a two-step process. In the first step, depending on the concentration of initial whey proteins, the denatured whey proteins are aggregated. These complexes then partner with the casein micelles during the lengthy heating (Corredig & Dalgleish., 1996). The major interaction appears to involve the thiol-disulfide exchange reactions between the denatured β-Lg and κ-casein at the micelle surface.(ii) As suggested by Morr (1985) first, β-Lg denatures at high temperatures and then interacts with casein. While α-La denatures and binds with filaments of β-Lg at a higher temperature (90°C/10 min). If the heat treatment is slight, the unfolded protein can refold into native structure.

Age gelation is another widely studied problem resulted during storage of UHT processed milk (Burton, 1978; Kosaric et al., 1981). Age gelation occurs more readily in concentrated milk than in single-strength milks. Its mechanism remains unresolved as to whether the cause is biochemical or

physicochemical. Lorient (1979) proposed the possibility of residual or reactive proteases are the cause. The effect of heat on milk components is summarised in Table 6.

Fouling is one of the most significant processing limitation factors that affect raw milk composition during thermal treatments (Changani, Belmar-Beiny, & Fryer, 1997; Gotham, Fryer, & Pritchard, 1992). During the heat treatment, two type of fouling occurs. Type A fouling take place at low temperature (less 100°C) and type B fouling which occurs at high temperature (over 120°C) (Burton., 1968). Milk fouling can reduce the efficiency of heat transfer, and increases pressure drop which means more heat is needed, which increases processing costs (Bansal. et al., 2009). The chemical composition of the dairy fouling deposit depends on the processing conditions and the level protein content and the level of mineral content.

2.5.4.2.-Effect of heat on milk pH

The pH of milk decreases with increasing temperature and time (Van Boekel et al., 1989). The change of pH in milk appears to increase with increasing protein concentration (Van Boekel et al., 1989). The decrease in acidity resulted in a loss of CO_2, resulting in a loss of H^+ ions and formation of Ca phosphate (Lin, Lewis, & Grandison., 2006) as shown in the equation below.

$$3Ca^{++} + 2HPO_4 \rightarrow Ca_3(PO4)_2 + 2H^+$$

Milk pH is the most important factor that is affected during heat treatment of milk. The heat coagulation time (HCT) of most milks increases the pH values to its maximum 6.7, while drop down the pH to its minimum 6.9; milk stability increases at higher pH value.

As noted by Van Boekel et al. (1989) milk pH does not change linearly with extended heating time. It starts with a quick decrease in pH during the first two minutes of heating; then the pH drop more slowly and linearly with time (Van Boekel et al., 1989). The drop of milk pH is linked to the development of organic acids, mainly formic acid, from lactose upon heating in the presence of oxygen and precipitation of primary and secondary Ca phosphate as tertiary phosphate with the release of H^+ (Pyne & McHenry., 1955).

2.5.4.3-Effect of heat in milk minerals (salts)

The heat-induced changes in the milk salt system is either reversible shift in salt balance by changes in temperature or irreversible shift in salt balance. During heat treatment of milk, the differences and concentration in temperature adversely affect salt balance. Heat-induced coagulation is one of the problems raised during heat processing of milk, its mechanism still has not been outlined but the role of excess Ca ion and shifts in ionic equilibrium are involved (Fox & Morrissey., 1977; Morr., 1975).

The solubility of Ca phosphate decreases with increasing temperature and decreasing pH. When milk is heated to 120°C, the solubility of Ca phosphate decreases at a pH 6.8 (Dalgleish., 1989)

The dissolved or soluble Ca and phosphate during heating is transferred to the colloidal state to form colloidal micelles of caseinate phosphate. The colloidal of the soluble Ca and phosphate resulted by heat treatment causes wide changes in the structure of the micelles.

Dissolved Ca, and phosphate tend to revert to the original system, but it is not completely transferred to the original structure after heat treatment. At the same time aggregation of the caseinate-phosphate micelles may occur. The minerals concentration in heat-treated milk is lower than that of raw milk.

The ionic Ca concentration of milk depends on the initial pH of milk before heating. Soluble Ca or ionic Ca activity does not affect when milk pH decline during heating while the decrease of pH is accompanied by an increase in ionic Ca concentration. Soluble Ca or ionic Ca concentration can be restored when milk is cooled (Van Boekel et al., 1989).

2.5.4.4-Maillard reaction

The Maillard reaction (nonenzymatic glycation) is a complex chemical reaction that takes place between amino groups and reducing sugars (lactose) during milk processing (heat treatment) or storage. The amino acids groups are mainly lysine residues (Walstra & Jenness, 1984), while The reducing sugar in milk is lactose, a disaccharide of glucose and galactose.

It is much faster at a temperature above 100°C, which results in a change in colour and flavour as well as loss in essential amino acids (Manji & Kakuda, 1988). Lactose a disaccharide sugar is main carbohydrate of milk commonly referred as milk sugar. Reducing sugars (lactose) reacts with the free amino acids of protein to produce early Maillard reaction (Mauron., 1981). It is very substantial for foods because it affects the quality. The Maillard reaction is sometimes subdivided into three stages: (I) Early Maillard reaction, which consists of condensation of the reducing sugar with the amino group and leads,(ii) Advanced Maillard reaction, which consists of the break- down of the Amadori product into numerous fission products of the sugar-amino compound. (iii) Final Maillard reaction consists of the condensation of amino com- pounds and sugar fragments into polymerised protein and brown pigments (Mauron., 1981).

Maillard reaction has considerable consequences for milk and milk products:

(i) The loss of nutritive value due to blockage of lysine residues (Finot, 1990), reduce digestibility and inhibition of enzymes (Friedman, 1996)

(ii) The formation of flavour compounds (Danehy., 1986)

(iii) The formation of antioxidative compounds (Bressa, Tesson, Dalla Rosa, Sensidoni, & Tubaro., 1996).

(iv) The formation of mutagenic (Shibamoto, 1982), as well as anti- mutagenic (Kato, Lee, Van Chuyen, Kim, & Hayase., 1987) and anticarcinogenic compounds (Aeschbacher., 1990).

(v) The formation of antibacterial compounds may be formed (Einarsson, Snygg, & Eriksson., 1983).

(vi) Antigenicity of heated cow's milk may be less for people allergic to cow's milk (Friedman, 1996).

(vii) The polymerisation of milk proteins because of the Maillard reaction (Zin-El-Din, Aoki, & Kako, 1991).

(viii) The development of brown colour due to melanoidin (Patton., 1955).

Chapter 3

Material and Methods

3.1-Materials: -

Milk used in the trials are FWM, FSM, and PSM. Four liters of FWM were collected from Lincoln University dairy farms [about 1400 cows are involved in the research; Ashley Dene farm (ADRS) 450 cow, Lincoln University dairy farm (LUDF) 500 cows and Lincoln University research dairy farm (LURDF) 450 cows] fortnightly for nine months (from April 2017 to February 2018). Spring (October, November and December), summer (January, February and March), autumn (April, May and June) while winter (July, August and September). Samples were mixed well before use. The supplier provided milk fat and protein contents for these fresh raw milk samples. Fresh milk was centrifuged at rate of 3000 x g for 30 min using a high-speed Beckman j2-MI centrifuge with rotary JA-10 (Backman, J2-MI, US Florida) and the cream (fat) layer was removed carefully yielding skimmed milk. Each test for each sample was performed in duplicate (triplicated measurements were done when a coefficient of variation is more than 20%). The experiments were conducted at room temperature 20 ±2°C. 10 mM stabilizer salts di-sodium hydrogen phosphate (DSHP) (AnalaR, Pool BH15 1TD, England), tri-sodium citrate (TSC) (Fisher, UK), sodium dihydrogen phosphate (SDHP (AnalaR, Pool BH15 1TD, England),) were added to FSM to improve/alter heat stability (Sweetsur & Muir, 1980). The sample scheme is presented in Table 7. Sodium azide (Fisher, UK) solution was used as a preservative (at concentration of 0.03%(W/V) (Gallier, Ye, & Singh, 2012). Sodium azide is a potentially explosive and highly toxic inorganic salt.

3.2-Methods:

3.2.1-Sample preparation and heat treatment:

Physicochemical, rheological and stability tests such as pH, Ca^{++}, buffering capacity (BC), viscosity, sedimentation rate, ethanol stability (ES) and PSD for FWM or FSM was conducted within 24 hours of milk collection (stored at 4℃ before analysis). Compositional analysis including total phospholipid content, protein composition, fatty acid composition and mineral composition were tested within a year time. Therefore, the corresponding samples were kept in a freezer (-20℃) before using. Before commencing any tests, milk was mixed well in a four-liter container. Samples were labelled with a seven digits number, including the year, month and sample number. Example: 2017031, 2017 represent the year; 03 accounts for March and one shows that the sample is the first sample in the month. Assays were conducted on FWM, FSM, PSM samples.

Three liters of fresh milk was centrifuged at 3000 x g for 30 min using a high-speed Beckman j2-MI centrifuge with rotary JA-10 (Backman, J2-MI, US Florida) and the fat was removed yielding FSM. An

aliquot of FSM (220ml) was high sheared (11,000 rpm for 10 min, before and a second aliquot after heat treatment using a high shear mixer (Polytron, Polytron 3100D, Luzern).

Eight aliquots of 220 ml of FSM (including sheared samples) with or without stabilising salts were heat treated in a water bath temperature of 85℃ for 5min (Labropoulos, Palmer, & Lopez, 1981) (for sample details, refer to Table 7). The current heat treatment was chosen to mimic the effects of UHT treatment using bench scale water bath heat treatment. Two major concerns were taken into consideration: 1. whey protein denaturation rate; 2. Whey protein denaturation kinetics. Labropoulos et al. (1981) concluded that vat heat treatment of 82 °C for 5 min denatured 88% whey protein in milk. Such heat induced whey protein denaturation rate is equivalent to a UHT heat treatment of 149°C for 10s. Moreover, protein concentration was found to affect the kinetics of denaturation/aggregation of β-lactoglobulin regarding forming intermolecular S–S bridges at low temperatures, but such effects were not observed at 85℃ temperatures (Iametti, Cairoli, Degregori, & Bonomi, 1995; Wijayanti, Bansal, & Deeth, 2014). It is expected that both protein content and composition will change among and also between seasons. 85℃ is selected in the current research as the heating temperature.

Table 7: Sampling scheme for heat treatment (85° C/5min)

Sample	High shear applied	Addition of Stabilizing salt
1	Before heat	none
2	Before heat	10mM DSHP
3	Before heat	10mM TSC
4	Before heat	10 mM SDHP/DSHP (2:1)
5	After heat	none
6	After heat	10mM DSHP
7	After heat	10mM TSC
8	After heat	10mM SDHP/DSHP (2:1)

Ten mM of stabilisers will be added to FSM before treatments. Di-sodium hydrogen phosphate, TSC (Sweetsur & Muir, 1980) and a mixture of SDHP and DSHP (2:1) (Montilla & Calvo, 1997) will be added to individual samples (Table 7). Samples with non-added stabilising salts were considered as controls. PSM will be assessed for pH, Ca^{++}, sedimentation, colour and PSD (casein micelles) on day 1 and day 30 after processing.

3.3-Analytical methods and references

3.3.1-Fat

The percent fat was provided by the supplier. It was determined by Babcock fat analysis method by using 92% sulphuric acid (H_2SO4) specific gravity 1.825. An 18.0g (17.6 ml) of raw milk at 20℃ is transferred to glassware of milk bottle, 17.5 ml H_2SO_4 was added to the sample. Rotate the bottle

between thumb and fingers while adding acid to wash milk from neck and mix thoroughly 2 min. Centrifuge the bottle containing the sample and H_2SO_4 for 5 min. Then measure the length of fat column with dividers from top of upper meniscus to the bottom of lower meniscus (Marshall, 1993).

3.3.2-Protein

The percent proteins were provided by the supplier from Lincoln University dairy farms. It was determined by testing for total Nitrogen content by the Kjeldahl method. The percent of nitrogen estimated was multiplied by factor (6.38) to get protein percentage (Horwitz & Latimer, 2000).

1-Digesting

0.1-0.2 g of Raw milk sample is used for the determination of protein. 0.1-0.2 g of raw milk sample were added to Kjeldahl tube,5ml of H_2SO_4 is added to the tube that contain the sample. The tube that contain the sample and H2SO4 heated on a digestion system 20, model 1015 digester.

$$\text{Raw milk} + H_2SO_4 \longrightarrow (NH_4)_2SO_4$$

2-Distilation

Add NaOH to the $(NH_4)_2SO_4$.

$$(NH_4)_2SO_4 + NaOH \longrightarrow 2NH_3 + Na_2SO^4 + 2H_2O$$

3-Titration

The 2NH3 were titrated using 0.1N of any of these acids HCl.

$$2NH_3 + HCl \longrightarrow NH_4Cl$$

The percent protein was calculated using the milk protein conversion factor 6.38.

3.3.3-Total solids percent (TS %)

Total solids content of milk is determined by a direct oven drying method. Blank containers were weighed before adding samples (W0). Milk sample + containers were weighed before putting into an oven (W1), samples left in overnight oven at 105℃, dry samples were kept in a desiccator overnight and weighed with the container (W2). All weights were taken using an analytical balance and read to the nearest 0.01 g. Total solids content is the weight of dried product residue expressed as a percentage of original product weight. The following formula gives the calculation of total solids.

Blank container weight= W0

Milk sample + container weigh= W1

Dried residue+ container weight=W2

$$\text{Total solids percent (TS \%)} = \frac{[(W2) - (W0)]}{[(W1) - (W0)]} X\ (100)$$

All samples were analysed in duplicate, and the average taken as the final value for that sample.

3.3.4-pH

The pH was measured using a pH meter (Mettler Toledo, Seven Easy s20, China). The pH of fresh milk was measured at 20°C (room temperature). The pH meter was calibrated using buffers of the standard solutions pH 4 and 7 using a pH probe. The pH of bovine milk varies between 6.6 and 6.8 (Chavez et al., 2004; White & Davies., 1958). Small decreases in pH (0.1- 0.25 units) have a large impact on the running time of the heat exchanger. Of course, there may be other factors than pH that can influence running time or product stability, such as a high total bacterial count, or incorrect salt balance.

3.3.5- Free calcium ion (Ca^{++})

Milk Ca^{++} concentration was measured using an ion meter (LAQUAtwinB-751, HORIBA, Japan) (Chen, Grandison, & Lewis., 2015; Silanikove, Shapiro, & Shamay, 2003).The instrument was calibrated in the millivolt (mV) output mode with solutions of 150 and 2000 mM. Ca^{++} concentration in bovine milk ranges from 2.0 to 2.3mM (Christianson et al., 1954).

3.3.6-Ethanol stability

Ethanol stability was measured by adding 2ml of fresh milk to a Petri-dish. The petri-dish was weighed. Ethanol 99% (density 0.789) was added dropwise until a precipitate appeared, then weight the petri dish to find the amount of ethanol added to the milk in grams (Xg). The coagulation of alcohol with milk was calculated according to Chen, Grandison, and Lewis. (2012). The amount of ethanol added is less than the sample volume. Add water till the added ethanol and water is equal to the sample volume. If the amount of ethanol added is equal to sample volume, do not add any water.

$$Xml = Xg / 0.789\ g$$

$$Yml = 2ml - Xml$$

$$Z = (Xg \times 0.99 / y + Xg) \times 100$$

Xml – Addition of Ethanol

Xg – is the amount of ethanol in gram in the sample

Yml –the amount of water added to the sample to equal to the sample volume

Z- Ethanol stability of raw milk in percentage

3.3.7-Buffering capacity (BC)

The buffering capacity of milk and milk products is an important physio-chemical characteristic that resembles to the ability of the products to be acidified or alkalinised. The value of the buffering capacity depends on several constituents of the product such as inorganic phosphate, citrate, organic acids and proteins. Buffering capacity was measured by adding 4.0 ml 0.1M HCl solution to 25 ml

sample and left for 1 h at room temperature. The milk pH is measured before adding HCl and after an hour of adding the HCl. The difference in pH of the sample before and an hour after adding 4.0 ml 0.1M HCl was read and this is the buffering capacity and expressed as pH units. It is calculated by the formula given below (Chen et al., 2015).

A buffering capacity value at each pH can be determined graphically by measuring the slope of the tangent. Van Slyke (1922) defined a dB/dpH ratio to calculate buffering capacity in a defined pH range. This ratio expresses the relationship between the increases of acid or base (B) added and changes in pH. Normality of HCl is number of hydrogens multiplied by the molarity (0.1M *H+)

dB/dpH= Volume of HCl added Normality of the HCl/Volume of sample(milk) ×pH change

3.3.8- Particle size distribution (PSD)

The overall particle size distribution, volume-weighted mean diameter (D 4,3) and surface area-weighted mean diameter (D 3,2) were measured at Plant & Food Research Center using mastersizer 2000 (Mastersizer 2000, Malvern, UK) for both FWM (milk fat globules) and FSW/PSM (casein micelles) (Ye, Singh, Taylor, & Anema, 2002; Zheng, Jiménez-Flores, & Everett, 2013). For skim milk samples, to avoid interference of remaining fat, only particles with diameters between 0.02 and 0.83µm will be included when estimating the volume-weighted diameter of the casein micelles (Gustavsson et al., 2014).

3.3.9-Dry sedimentation percent (DS %)

This is a modified method of Chen et al. (2012). Briefly, sediment was measured following the centrifugation method described below. A sample was well shaken, and 48 g of sample accurately weighed and poured into a calibrated tube and centrifuged at 2,760 × g for 30 min. After the supernatant is removed, the sediment will be oven-dried at 105°C to constant weight to determine its dry weight percentage.

Dry sediment percent (DS %) = (B/A) ×100

A =weight of sample in grams

B =weight of dried pellet in grams

3.3.10-Total phospholipid

For each milk sample, 200 mg milk was frozen at -20 °C until further use. Total phospholipid content was measured using phospholipid assay kit (Sigma0-Aldrich, MAK122).

Preparation of standards

Step one:

24 µL of 2 mM of Phosphatidylcholine add 216 µL of RO water to prepare a 200 µM standard solution.

Step two:

Transfer 0 µL, 30 µL,60 µL and 100 µL of standard solutions to 1.5ml Eppendorf tube and mix the(vortex). Add RO water to each of the standard solution to bring the volume in each tube to 100 µL.

Step three:

Transfer 20 µL of standards of 0 µL, 30 µL,60 µL and 100 µL into separate wells of 96 well plate in addition to 20 µL of the sample and one well of 20 µL of RO water.

Step four:

Sample preparation

A-Enzyme mix is prepared by mixing 85 µL assay buffer to 1 µL to the enzyme mix to 1 µL PLD enzyme and 1to µL of dye reagent. Multiply the volume by the number of samples.

B- A blank sample is prepared by mixing 86 µL assay buffer to 1 µL enzyme mix and to 1 µL dye reagent. Multiply the volume by two.

Step five:

Add 80 µL of the appropriate mix to each of the wells that are filled with 20 µL and add 80 µL of the sample blank to a different well.

Step six:

Mix well using a horizontal shaker or by pipetting and incubate the reaction 30 minutes at room temperature. Protect the plate from light during the incubation.

Step seven:

Measure the absorbance of the samples and standards at 570 nm for the colourimetric assay or the fluorescence intensity (λex = 530/λem = 585 nm) for the fluorometric assay.

3.3.11-Protein composition

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) is a commonly used method to identify protein composition of FSM due to its high resolution of protein separating by size and charge. SDS-PAGE will be used for analysing and quantifying the protein composition using densitometry technique (Ye et al., 2002; Zheng et al., 2013). For each milk sample, 5ml fresh skim milk (3000g for 30 minutes) was frozen at -20 °C until further use. The samples were defrosted before analysis. FSM prepared by centrifuging the fresh milk at 3000g for 30 minutes. The supernatant (skimmed milk) was transferred to a new centrifuge tube and stored at 4°C until use. Before the electrophoresis, 2ml of skim milk was diluted with 6 ml of Reverse Osmosis (RO) water. For total 4 samples, 26 µl of the diluted skim milk sample, the following were added.

Sample buffer of 10 µl (the sample buffer used was Nu PAGE® LDS Sample Buffer (4X) and 4ul of 5 % DTT. After adding all the above solutions to the diluted skimmed milk sample, the mixture was heated in a water bath of 70°C for 10 minutes.

Preparation of the Running solution: The Running solution was prepared by adding 50 ml MES (2-[N-morpholino] ethane sulfonic acid) to 950 ml of RO water and mixed well.

Gel: The Gel used was 4 – 12% Bis-Tris Gels which can be stored at 4 °C for a maximum period of 12 months. These are the pre-packed polyacrylamide gels designed for optimal separation and resolution of small to medium sized proteins (1.5 – 300 kDa) under denaturing gel electrophoresis conditions. The Criterion Cell (Bio-Rad) was attached to the Midi Gel Adapter to the Midi Gel Cassette. The comb was removed and rinsed the gel wells three times using 1X Running Buffer. The white tape near the bottom of the gel cassettes was removed, and the gels were placed in the gel running tank. Then, the gel wells were filled with the same 1X Running Buffer that was used in the Upper Buffer Chamber. Finally, 10ul of the sample (prepared as above) was loaded to each well. The molecular weight marker was then added to the 1st well. The Life Technologies power supply was installed with the Novex power supply adapters (Catalog number ZA 10001). The MES Running Buffer, run at 200 V constant for about 40 minutes with the XCell4TM or until the protein has migrated to the bottom of the gel. The gel was then removed from the buffer, and the gel was stained (using a blue stain). Covered the gel with foil and transferred to a shaker while in the buffer to wash off excess stain so that the proteins become more visible. Finally, it was scanned using Canon Scanner of model (CS9000F Mark II), and the protein concentration in the milk sample was calculated using the formula given below.

Protein Concentration = Protein % in milk × volume of milk (2ml) / amount of RO water added (6ml; diluent)

The value from above is divided by the total volume of sample buffer (10ul) and DTT (4ul) = 14ul. This gives the protein concentration the prepared sample.

3.3.12-Fatty Acid Compositions (FA)

For each milk sample, 5ml fresh whole milk was frozen at -20 °C until further use. FA was measured using a Shimadzu GC-2010 gas chromatograph (Shimadzu GC-2010, Japan) with AOC-20i auto-sampler, with column Varian CP7420, fused silica, and 0.25 mm x 100 m, 0.2 um film thickness (Folch, Lees, & Stanley., 1957)

Sample Preparation Protocol

Make sure to use dry methanol for NaOH/MeOH solution. (Use molecular sieves to dry the methanol, then use anhydrous sodium sulphate to test for dryness.)

Procedure:

1. Weigh empty Kimax tubes, record
2. Pipette 1ml (500ul) of sample into each Kimax tubes and weigh
3. Freeze dry the samples in a Kimax tube (1-2 days), then weigh

(Step 2 & 3 can be replaced by using already freeze-dried samples and weigh 0.15-0.17 g (0.07-0.08g) of samples into each tube)

4. Add 100uL (50ul) of internal standard (C21:0 ester, ~5mg/ml) into each tube.
5. Add 900uL (450ul) of Heptane into each tube
6. Add 4.0ml (2.0ml) of 0.5M NaOH/dried methanol to each tube
7. Fill the tube with Nitrogen (avoid oxidation), screw the caps tight, then carefully vortex (try avoiding solids sticking on the wall)
8. Incubate in Heating Block (water bath) at 50°C for 15 minutes
9. Cool the tubes to room temperature on the bench, vortex again
10. Add 2.0ml (1.0ml) of Heptane and 2.0ml (1.0ml) distilled water, cap and vortex
11. Centrifuge at rate1500g for 5 minutes
12. Transfer top layer into another tube. Add 2.0ml (1.0ml) of Heptane to the original tubes, vortex and centrifuge for 5min @ 1500g again
13. Pool top layer in the second tube, mix and add small amount of Na2SO4 to remove residue water, sub-sample to a GC vial and store in -20°C until GC analysis

Chemicals used:

- ISTD (C21:0 easter): ~5mg/ml in Heptane
- 0.5M of NaOH in Anhydrous Methanol (20g NaOH in 1L methanol)
- Nitrogen
- n-Heptane

3.3.13-Mineral compositions

For each milk sample, 5ml milk was frozen at -20 °C until further use. The total mineral composition is measured using Inductive Coupled Plasma-Optical Emission Spectrometers (ICP-OES) (Varian -720 ICP-OES, USA). It is one of the fastest analysis to simultaneously determine several minerals in milk samples, not only at high concentrations. This technique has good sensitivity and accuracy in determining minerals such as Ca, P, Na, Mg, K, Mn, Zn and Fe (Sanders, 1931).

3.3.14-Colour

PSM is scanned using chroma meter. Hunter L*, a*and b* value will be determined using a chroma meter (Minolta, CR-210, Japan) on day 1 and day 30 after processing (Schamberger & Labuza, 2007). Total change in colour is calculated using Hunter Lab (1996) equation.

Milk colour is measured for its Hue and chroma.

Hue (angle)

Hab = tan-1 (b*/a*)

Chroma (magnitude) C*ab = [a*2 + b*2]1/2

Total colour change (ΔE) was calculated with equation $\Delta E=\sqrt{(\Delta L2+\Delta a2+\Delta b2)}$

3.4- Milk processing

3.4.1- Skimmed milk

Three liters of fresh milk was centrifuged at 3000 x g for 30 min using a high-speed Beckman j2-MI centrifuge with rotary JA-10 (Backman, J2-MI, US Florida) and the fat was removed yielding FSM. An aliquot of FSM (220ml) was high sheared (11,000 rpm for 10 min, before and a second aliquot after heat treatment using a high shear mixer (Polytron, Polytron 3100D, Luzern).

3.4.2- Heat treatment

Before any treatment stabilizing 10mM salts of Di-sodium hydrogen phosphate (DSHP), Tri-sodium citrate (TSC), and Sodium hydrogen phosphate (SDHP) were added to 8 sample of 220ml of FSM (Sweetsur & Muir, 1980). 2 out of the 8 sample contained a mixture of SDHP and DSHP in (2:1) ratio. 4 samples were heat treated at 85 ℃ for 5min in a hot water bath and then high sheared using a high shear homogenizer at a rate 11.0x1000 rpm for 10min, while the other 4 samples were high sheared using a high shear homogenizer at a rate 11.0x1000 rpm for 10min and then heat treated at 85 ℃ for 5min in a hot water bath. Know molecular or mass weight of each salt calculate the grams added to the sample.

Heat exact amount of milk (220ml) for each treatment; Sodium azide was added to treated milk samples (final sodium azide content is 3% w/v). Sodium azide is a highly toxic inorganic salt that dissolves readily in aqueous solutions. It is frequently used in research as a preservative to maintain perishable chemical reagents. The treated (homogenised and heated) milk samples containing 3% v/v sodium azide are kept at ambient temperature for shelf life study (note: sodium azide is toxic, the samples cannot be kept at food grade labs/areas)

The amount of stabiliser added is calculated as follow:

(Amount of milk used in L) *(0.01M) *(Molecular weight of salt)

Molarity= Mole/volume in L

Sodium citrate (TSC) molecular weight= 294.09 g/mole

Di-sodium hydrogen phosphate (DSHP) molecular weight 177.99 g/mole

Sodium hydrogen phosphate molar mass (SHDP): 137.99 g/mole

3.4.3-High shear homogenization of skimmed milk

After stabilizing 10mM salts of Di-sodium hydrogen phosphate (DSHP), Tri-sodium citrate (TSC), and sodium hydrogen phosphate (SDHP) were added to the samples of FSM (Sweetsur & Muir., 1980), 4 samples were heat treated at 85 ℃ for 5min in a hot water bath and then high sheared using a high shear homogenizer at a rate 11.0x1000 rpm for 10min, while the other 4 samples were high sheared using at the same rate at same temperature for the same time as below in figure 4.

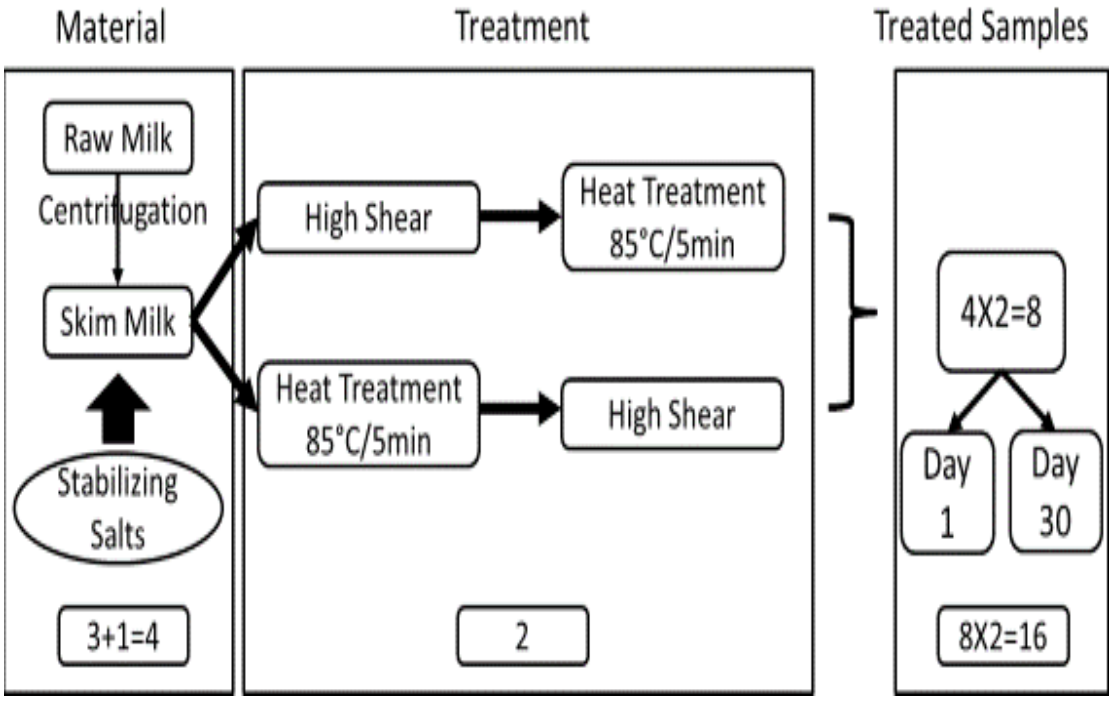

Figure 4: Raw milk processing flow chart

3.5-Statistical analysis

For Statistical analysis, Minitab 17 was used. One-way ANOVA statistical analysis was performed to reveal the significance of each of the assayed parameters between samples; Multivariate analysis (principal component analysis, PCA) was used to determine the impact of seasonality on a series of attributes of fresh and treated milk samples collected during different months and seasons.

Chapter 4

Raw milk analysis

4.1-Introduction

Raw milk samples collected from Lincoln University dairy farm between April 2017 to February 2018 were investigate if there was seasonal varies in its composition. The seasons are, Spring (October, November and December), summer (January, February and March), autumn (April, May and June) while winter (July, August and September). All investigations are held under standardised conditions. This paper presents the results for properties of the raw milk collected from Lincoln University dairy farm fortnightly for nine months. Samples were mixed well before use. The supplier provided fat and protein percentage. One-way ANOVA and Multivariate analysis (principal component analysis, PCA) were used to study the obtained data.

4.2-Chemical properties of raw milk

4.2.1-Fat percentage (F %)

Table 8: Seasonal variations of chemical composition and the physical properties of fresh raw milk and fresh skimmed milk collected over the period March 2017 to February 2018 (Results are mean± Standard deviation).A=autumn, SP=spring, S=summer and W=winter

properties	Spring	Summer	Autumn	Winter	S. Variation
Protein (%)	3.7 ±0.1	4.1 ±0.3	4.8 ±0.3	4.1 ±0.5	A>W, S, SP & SP>W, S
Fat (%)	4.7 ±0.1	5.1 ±0.4	6.1 ±0.2	5.4 ±0.6	A>W, S, SP & SP>S
TS (%)	14 ±1	15 ±1	15 ±2	15 ±1	NS
Ca^{++} (mg/L)	92 ±7	99 ±13	115 ±15	94 ±23	A>W, SP
pH	6.69 ±0.04	6.67±0.04	6.70 ±0.03	6.72 ±0.05	A>S
BC	0.019±0.01	0.020±0.1	0.021±0.002	0.019±0.002	A>SP, W
ES (%)	21.4 ±8.2	20.3±7.5	45.6 ±10.7	33.6 ±7.6	A>W, SP, S &W>SP, S
D (3,2)	0.25 ±0.02	0.26±0.04	0.24 ±0.03	0.26 ±0.02	NS
D (4,3)	2.91 ±0.22	2.92±0.28	2.4 ±0.4	3.1 ±0.3	A>W, S, SP & W<S<SP
DS (%)	0.35 ±0.12	0.55±0.12	0.54 ±0.18	0.21 ±0.21	S, A>W, SP
Total PL	3.1±0.2	3.3±0.2	3.3±0.1	2.8±0.5	S>W

As it is recorded in Figure 6, the investigation for fat composition showed significant variation ($p<0.05$) between different seasons. The highest average fat content of 6.15±0.20% was recorded in autumn, while the lowest fat content was detected in spring with the of 4.73±0.09% as recorded in table 8, this finding contradicted to finding by (Fox & McSweeney, 2003).

Table 9: Monthly protein, fat and total Solid percentage of fresh whole milk over the period of March 2017 to February 2018 (Results are mean± Standard deviation).

Months	Protein	Fat	Total solid
October	3.69±0.01	4.78±0.01	14.82±1.23
November	3.73±0.12	4.65±0.12	13.79±0.35
December	3.76±0.01	4.77±0.04	14.29±0.79
January	3.89±0.02	4.81±0.06	14.34±0.15
February	4.08±0.16	5.13±0.19	14.84±0.47
March	4.57±0.00	5.89±0.00	17.70±0.28
April	4.66±0.06	5.96±0.09	15.86±0.81
May	4.99±0.17	6.31±0.21	13.67±3.73
June	4.72±0.17	6.19±0.12	15.70±0.27
July	4.73±0.07	6.16±0.01	16.17±0.17
August	3.99±0.17	5.15±0.36	15.05±0.25
September	3.69±0.03	4.85±0.12	13.63±0.44

Table 10: Monthly analysis of variance result of the chemical composition and the physical properties of fresh raw milk collected over the period March 2017 to February 2018 (Results are mean± Standard deviation)

Variables	Source	DF	Adj SS	Adj MS	F-Value	P-Value
Protein %	Month	11	10.23	0.93	75.85	0.00
	Error	34	0.42	0.01		
	Total	45	10.64			
Fat %	Month	11	18.32	1.67	70.34	0.00
	Error	34	0.81	0.02		
	Total	45	19.13			
TS%	Month	11	48.67	4.42	2.88	0.01
	Error	34	52.30	1.54		
	Total	45	100.97			
Ca^{2+}	Month	11	10201.00	927.30	6.87	0.00
	Error	34	4593.00	135.10		
	Total	45	14793.00			
pH	Month	11	0.04	0.00	3.00	0.01
	Error	34	0.04	0.00		
	Total	45	0.08			
BC	Month	11	4.00	0.36	5.33	0.00
	Error	32	2.19	0.07		
	Total	43	6.19			
ES%	Month	10	4672.00	467.19	6.19	0.00
	Error	31	2338.00	75.42		
	Total	41	7010.00			
D (3,2)	Month	11	0.02	0.00	2.11	0.05
	Error	32	0.02	0.00		
	Total	43	0.04			
D (4,3)	Month	11	4.00	0.36	5.33	0.00
	Error	32	2.19	0.07		
	Total	43	6.19			
DS%	Month	11	0.57	0.05	1.72	0.11
	Error	34	1.02	0.03		
	Total	45	1.58			

Autumn sample was different from all samples while winter was different to spring and autumn but similar to summer (Figure 6). The average fat content in summer was higher (5.16±0.43%) than spring and winter milk (4.73±0.09%,5.38±0.62%) but statistically showed no significant difference (Figure 6). The same analysis was carried by changing the factor from seasons to months; it noted a significant variation in fat content ($p<0.05$) within months of the season as in table 10. The samples collected during summer (January, February, and March), March samples recorded significant difference from other months of the same season. While in winter samples (July, August, and September) samples from July were observed to had considerable variation than the rest of the months of the same season (Figure 9). The highest fat content was recorded in May 6.31±0.21%, while the lowest was in November (4.65±0.12%) (Table 9).

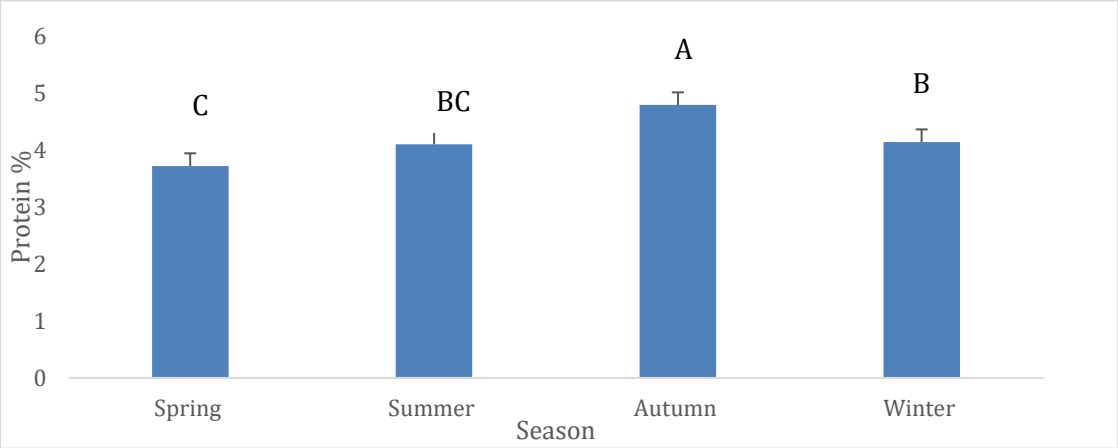

Figure 5: Seasonal protein percentage content of fresh raw milk collected over the period March 2017 to February 2018 (Results are mean and grouping). Means that do not share a letter are significantly different

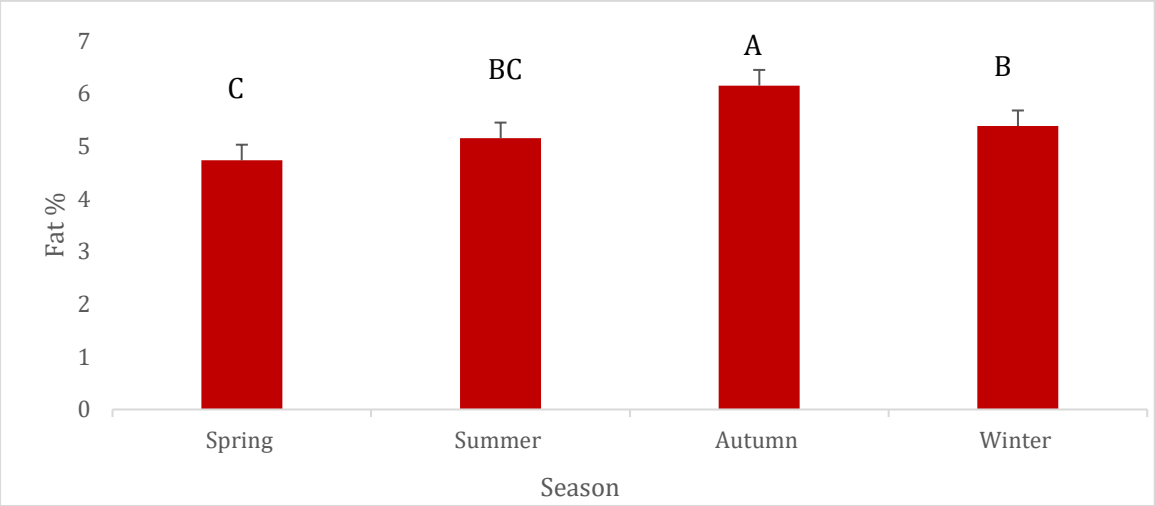

Figure 6: Seasonal variation of fat percentage of fresh raw milk collected over the period March 2017 to February 2018 (Results are mean and grouping). Means that do not share a letter are significantly different.

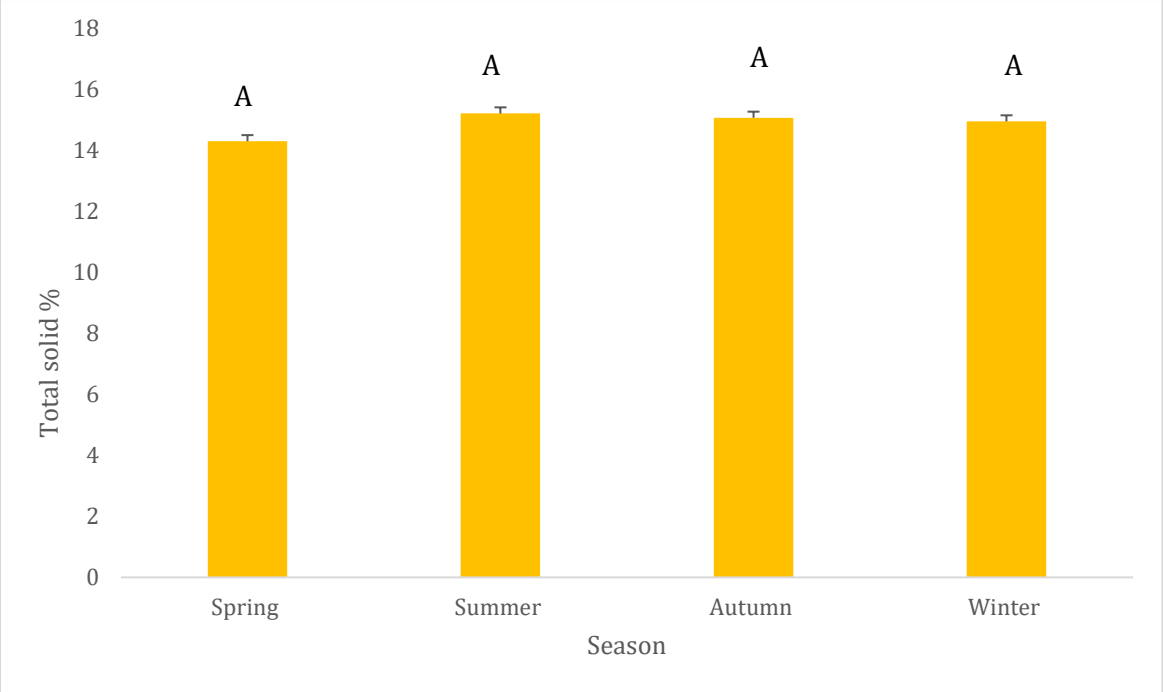

Figure 7: Seasonal variation of total solid percentage of fresh raw milk collected over the period March 2017 to February 2018 (Results are mean and grouping). Means that do not share a letter are significantly different.

4.2.2-Protein percentage (P %)

The study showed significant variation ($p<0.05$) in protein composition between different seasons (Figure 5). The highest protein level was 4.79±0.28% in autumn which is contradictory to the findings of (Fox & McSweeney., 2003), while the lowest was 3.72±0.07% in spring (Table 8). Autumn samples were different to all samples, while winter was different to spring and autumn but similar to summer as shown in Figure 4. The average protein content in winter was higher (4.14±0.47%) than to summer (4.10±0.28%) but statistically showing no significant difference. The same was recorded between summer and spring samples too (Figure 5) as they share the same alphabet. When the same analysis is carried by changing the factor from seasons to months, it is observed a significant variation ($p<0.05$) within samples of different months of the same season.

From the samples collected during summer (January, February, and March), March samples recorded significant difference than the other months of the same season. While Samples collected in autumn (April, May, and June), May samples recorded considerable difference than the additional months of the same season, all samples collected in winter (July, August, and September) was observed to have significant variation within the months as shown in figure 8. The highest protein content was recorded in May with the average of 4.99±0.17%, while the lowest was recorded in September and October with the average of 3.69±0.03% and 3.69±0.01% respectively as in Table 9.

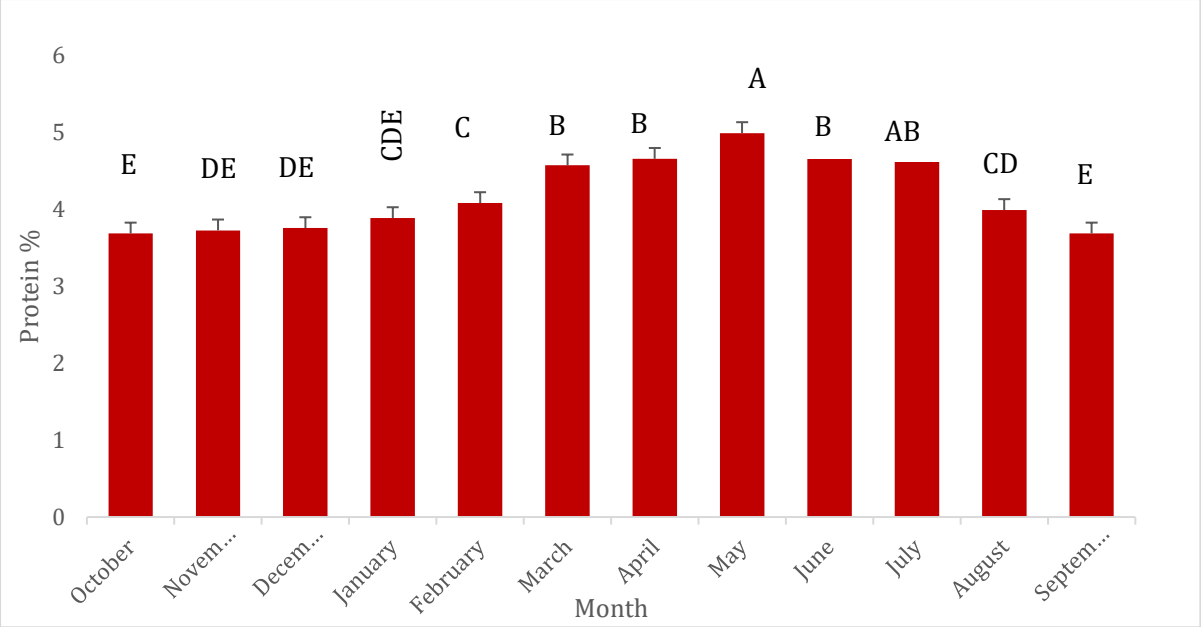

Figure 8: Monthly protein percentage (P%) of fresh whole milk over the period of March 2017 to February 2018 (Results are mean± Standard deviation). Means that do not share a letter are significantly different.

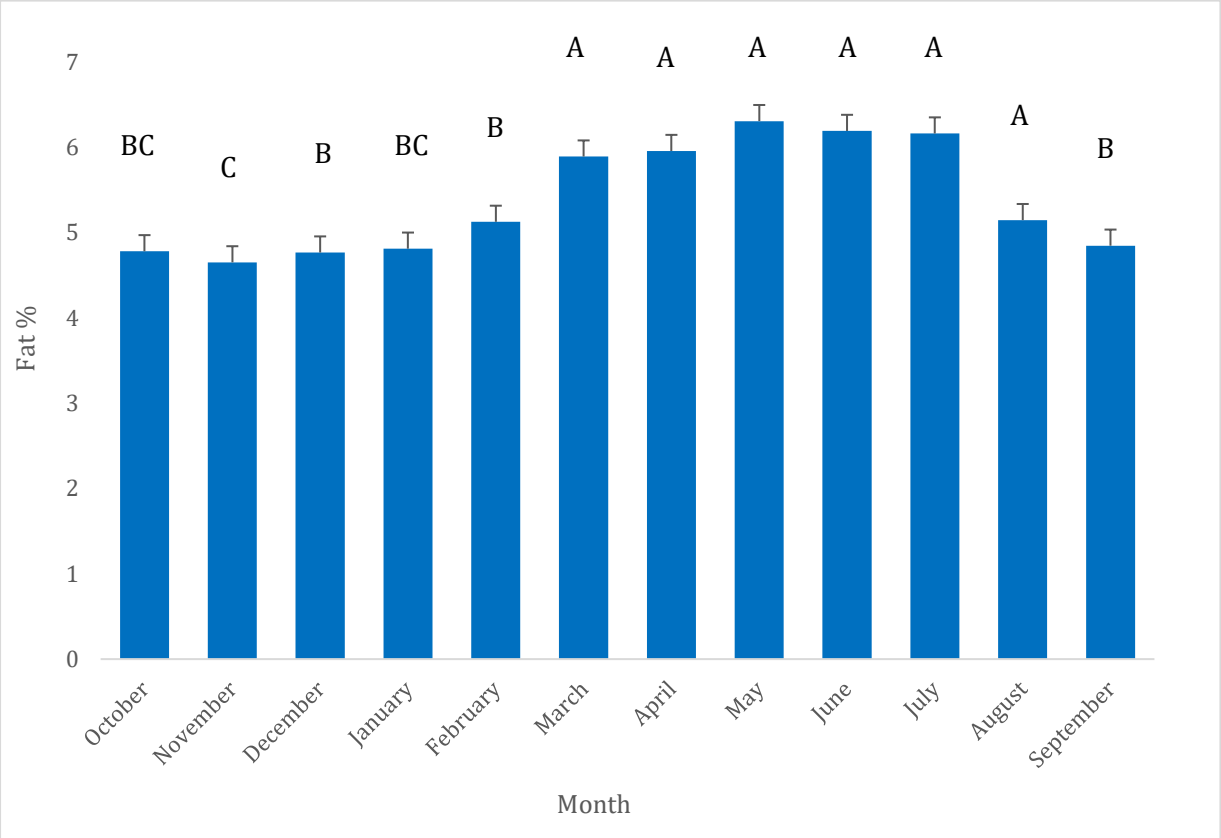

Figure 9: Monthly fat percentage (F%) of fresh whole milk over the period of March 2017 to February 2018 (Results are mean± Standard deviation). Means that do not share a letter are significantly different.

4.2.3-Total solid percent (TS %)

The investigation noted summer recorded the higher total solid, but no significant seasonal variation ($p>0.05$) as all seasons share the same letter as in Figure 7.

When the same analysis carried by changing the factor from seasons to months, the results noted that milk samples recorded significant variation ($p<0.05$) as in figure 5. The samples collected in March was significantly different from samples of May, September, and November as shown in Figure 10. The highest TS content was recorded in March with the average of 17.70±0.028%, while the lowest was recorded in September with the average of 13.63±0.44% as in Table 9.

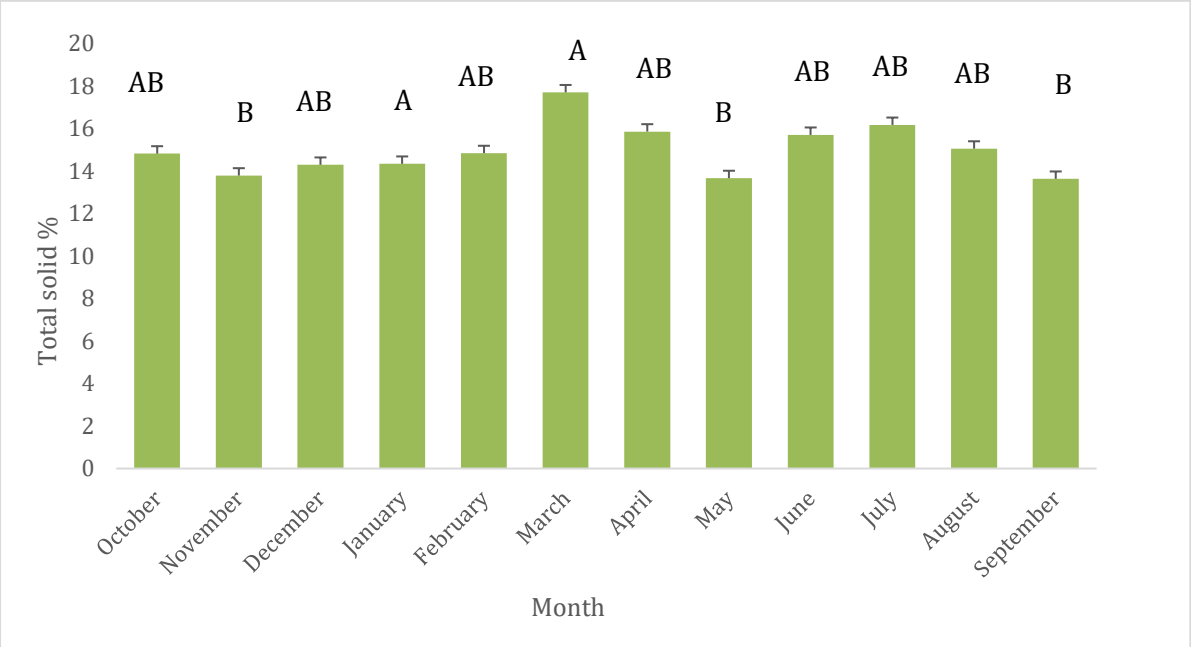

Figure 10: Monthly total solid percentage (TS%) of fresh whole milk over the period of March 2017 to February 2018 (Results are mean± Standard deviation). Means that do not share a letter are significantly different.

4.2.4-pH

The investigation held on pH of milk noted a significant variation ($p<0.05$) in pH value between the samples from different seasons. The result shows a difference between the pH in winter and summer (Figure 12). Highest pH noted in winter (6.72±0.05) and lowest in summer (6.67±0.04) as recorded in Table 8. Samples from spring, summer, and autumn show no difference to each other, and the same results for samples from spring, autumn, and winter (Figure 12).

When the same analysis carried by changing the factor from seasons to months, results noted milk samples collected during different months showed significant variation($p<0.05$) (Figure 13). Milk pH for samples collected in September recorded higher pH value (6.76±0.01); to milk from February; which recorded the pH value of 6.64±0.04 as shown in Table 10. February was found to be different to April and September, while September different to February and December.

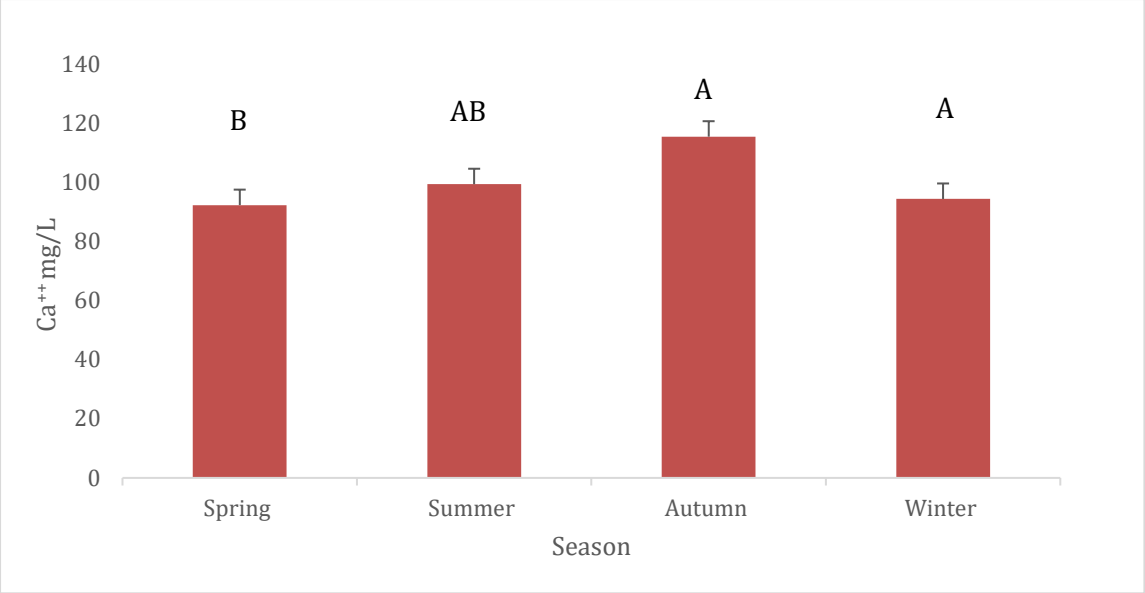

Figure 11: Seasonal difference of calcium ion (Ca^{++}) concentration of fresh raw milk collected over the period March 2017 to February 2018 (Results are mean and grouping). Means that do not share a letter are significantly different.

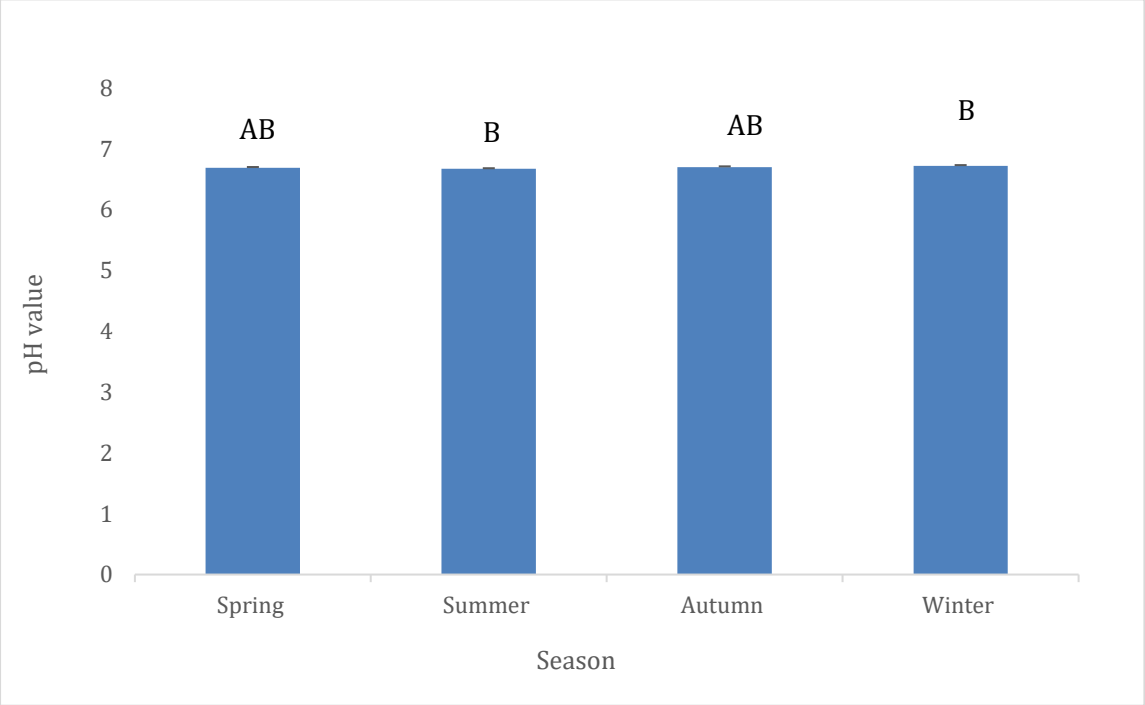

Figure 12: Seasonal difference of pH of fresh raw milk collected over the period March 2017 to February 2018 (Results are mean and grouping). Means that do not share a letter are significantly different.

4.2.5-Free calcium ion (Ca^{++})

The investigation recorded a significant variation ($p<0.05$) in Ca^{++} concentration between different seasons (Figure 11). The highest Ca^{++} level was noted in autumn 115.50mg/L, while the lowest was in spring 92.33mg/L as indicated in Table 8. There were no differences between samples collected in spring, summer, and winter, and some result was found to samples from summer and autumn

(Figure 7). Milk collected in autumn is different from winter and spring samples but no difference to summer, while no difference between samples from summer, spring, and winter (Figure 7).

Figure 13: Monthly variation of pH value of fresh whole milk over the period of March 2017 to February 2018 (Results are mean± Standard deviation). Means that do not share a letter are significantly different.

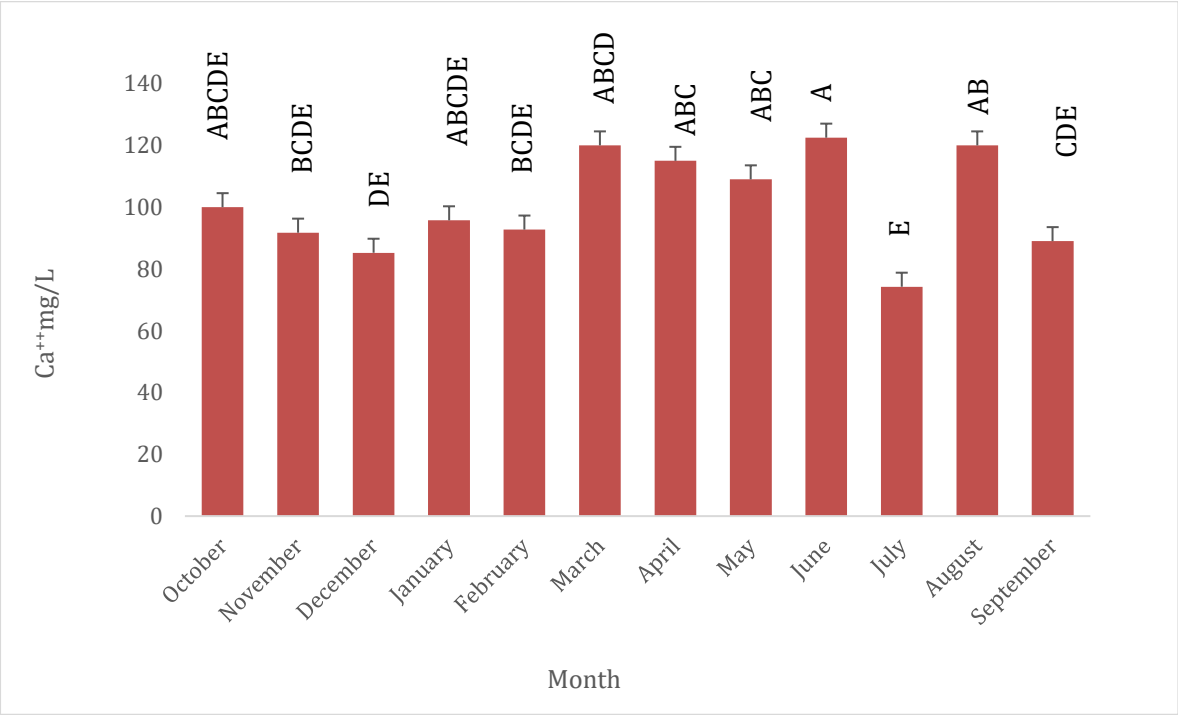

Figure 14: Monthly difference of calcium ion (Ca^{++}) concentration of fresh whole milk over the period of March 2017 to February 2018 (Results are mean± Standard deviation). Means that do not share a letter are significantly different.

When the same an analysis carried by changing the factor from seasons to months, the results noted a significant variation ($p<0.05$) within months of the season (Table 10). The samples from June was different from the July sample (Figure 14). June was also different to October, December and September while July different to March, April, May, and August.

Table 11: Monthly variation of pH value and calcium ion (Ca^{++}) concentration and ES% of fresh raw milk collected over the period March 2017 to February 2018 (Results are mean± Standard deviation).

Months	pH	Ca^{++} mg/L	ES %
October	6.69±0.04	100.00±0.00	21.22±9.11
November	6.72±0.01	91.75±4.50	22.84±12.19
December	6.67±0.04	85.25±15.56	20.10±2.99
January	6.695±0.01	95.75±11.50	17.48±7.82
February	6.64±0.04	92.75±6.70	23.08±6.98
March	6.69±0.00	120.00±0.00	
April	6.73±0.03	115.00±5.77	38.92±2.84
May	6.69±0.03	109.00±24.37	49.15±13.80
June	6.69±0.04	122.50±9.57	45.31±10.06
July	6.73±0.01	74.25±20.55	35.23±8.69
August	6.69±0.08	120.00±0.00	38.03±7.33
September	6.76±0.01	89.00±12.70	27.54±2.34

The Ca^{++} concentration for milk collected in June recorded the highest level 122.50mg/L while milk from July recorded the lowest level Ca^{++} (74.25mg/L) concentration (Table 11).

4.2.6-Buffering capacity (BC)

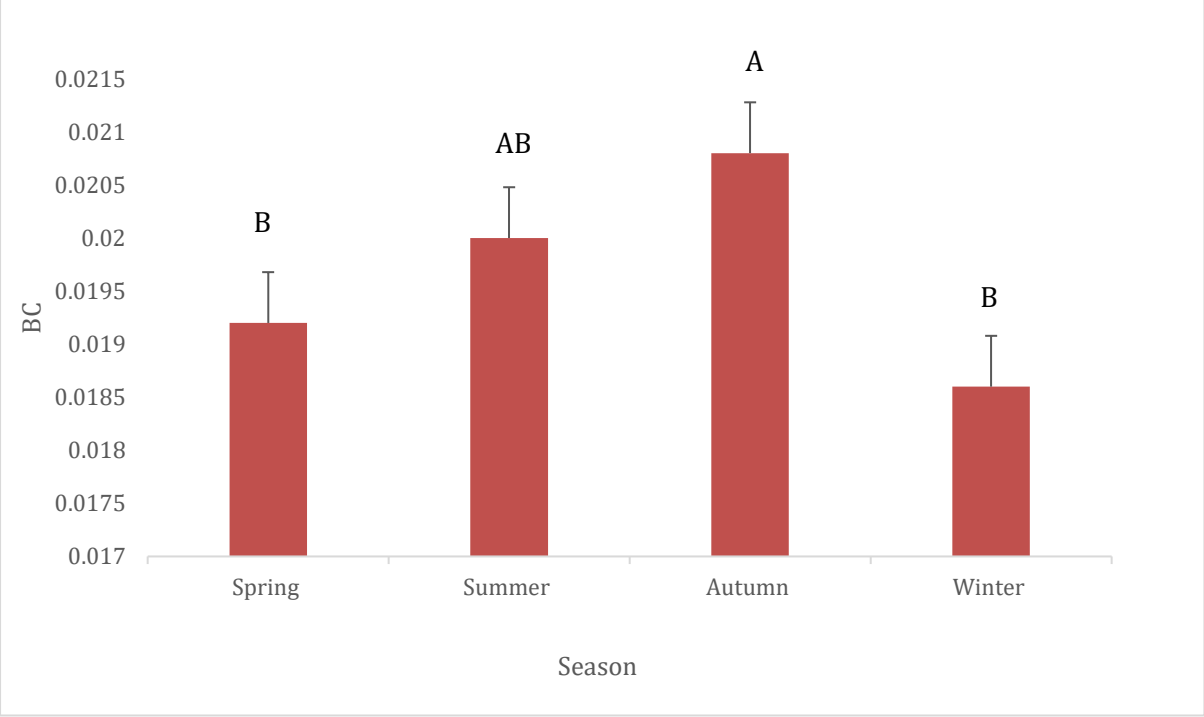

Figure 15: Seasonal buffering capacity (BC) value of fresh raw milk collected over the period March 2017 to February 2018 (Results are mean and grouping). Means that do not share a letter are significantly different.

The buffering capacity value at each pH can be determined graphically by measuring the slope of the tangent. The BC of fresh whole milk recorded a significant variation ($p<0.05$) between seasons as recorded in Figure 9. Autumn milk was significant than winter and spring while no difference from summer milk as in Figure 9.

Table 12: Monthly difference in buffering capacity (BC), particle size distribution (PSD) in µm (D [3, 2] - Surface weighted mean and D [4, 3] - Volume weighted mean) and dry sedimentation percentage (DS%) in fresh whole milk collected over the period March 2017 to February 2018 (Results are mean ± Standard deviation and grouping information).

Months	BC	D (3,2)	D (4,3)	DS%
October	0.020± 0.001	0.25± 0.03	3.02± 0.27	0.35± 0.12
November	0.020± 0.000	0.23± 0.00	2.70± 0.02	0.29± 0.16
December	0.018± 0.001	0.27± 0.02	3.02± 0.12	0.40± 0.10
January	0.020± 0.001	0.30± 0.06	3.12± 0.32	0.58± 0.13
February	0.019± 0.001	0.26± 0.02	2.84± 0.15	0.46± 0.08
March	0.020± 0.000	0.26± 0.01	2.68± 0.16	0.68± 0.00
April	0.020± 0.001	0.23± 0.04	2.28± 0.58	0.49± 0.24
May	0.022± 0.001	0.25± 0.02	2.61± 0.08	0.53± 0.13
June	0.021± 0.002	0.25± 0.01	2.52± 0.21	0.58± 0.21
July	0.020± 0.001	0.28± 0.02	2.89± 0.09	0.39± 0.24
August	0.017± 0.002	0.26± 0.02	3.40± 0.29	0.31± 0.15
September	0.019± 0.002	0.24± 0.02	3.11± 0.20	0.35± 0.26

The average value of BC, expressed as the slope of the tangent, was higher on autumn 0.0208±0.0015, while lower on spring and winter receded 0.0192±0.0014 and 0.0186±0.0020 respectively (Table 8). When the same analysis carried by changing the factor from seasons to months, it is observed a significant variation ($p<0.05$) within months. The sample from May was different from the August sample. The investigation noted the highest BC values in May 0.022 and the lowest in August of 0.017 as in table 12.

4.2.7-Total phospholipids (TPL)

The investigation recorded a significant variation ($p<0.05$) in the TPL between different seasons as shown in Figure 23. Winter was found to different to summer and autumn, while no difference between spring, summer, and autumn samples (Figure 23). The highest TPL in summer was 3.34 µL/M while the lowest TPL recorded in winter was 2.79 µL/M (Table 8). When the same analysis is done by changing the factor from seasons to months, it is observed significant variation ($p<0.05$) within months of the season. All the months found to have no difference except September. The highest TPL was recorded in March 3.49 µL/M while the lowest was in September 2.21µL/M.

4.2.8-Protein composition

This study recorded a significant variation ($p<0.05$) in the total whey protein and a-Casein between different seasons (Figure 16). The only difference recorded was between samples from winter and autumn in total whey protein, during summer and autumn in a-Casein. Winter recorded higher concentration of whey and casein while autumn recorded the lowest level of whey and casein (Table 13). No variation was noted for α-lactalbumin (α-LA), β-lactoglobulin (β-LG), total caseins, β- casein, and κ-casein (-CN) ($P>0.05$.)

Table 13: Seasonal variation of protein composition in KDa in fresh whole milk collected over the period March 2017 to February 2018 (Results are mean ± Standard deviation).

Proteins	Spring	Summer	Autumn	Winter
Whey	5350.68±2264.60	5183.29±1970.81	3343.06±1778.21	5568.50±1399.44
α-LA	2562.88±1543.50	2705.86±1493.77	1373.36±1390.75	2510.28±1111.83
β-LG	2787.80±1365.99	2477.42±1252.49	1969.69±926.03	3058.22±634.88
Casein	6298.60±2439.88	6394.64±2323.54	5201.40±2088.93	7289.00±2528.77
α-CN	2671.16±506.32	2958.56±1162.95	1914.43±847.90	2740.62±886.01
β- CN	1655.08±1804.15	1770.67±1571.52	1314.70±1319.06	2287.78±1318.00
k-CN	1972.36±1556.61	1665.41±1579.54	1972.27±1304.53	2260.57±1408.43

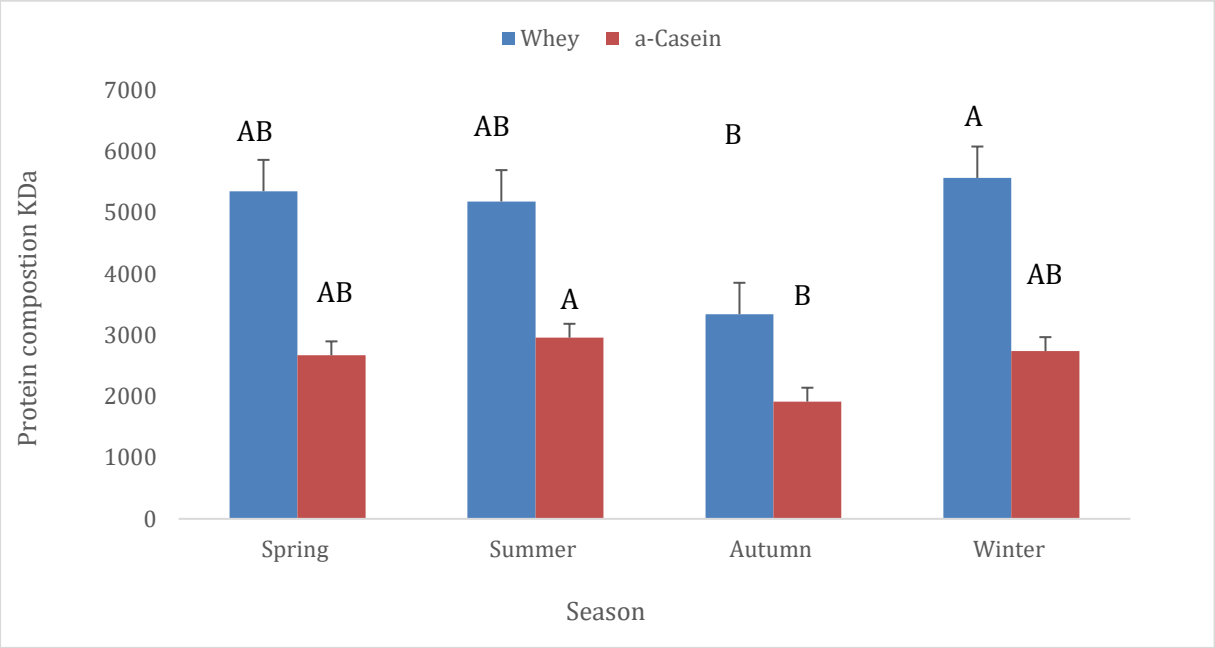

Figure 16: Seasonal total whey protein and a-Casein concentration in KDa in fresh whole milk collected over the period March 2017 to February 2018 (Results are mean and Standard deviation and grouping information). Means that do not share a letter are significantly different.

4.2.9-Fatty acids (FA)

The investigation was to see if there are seasonal variation in the percentage and the amount of palmitic acid (C16:00), stearic acid (C18:00), oleic acid (C18:1), Conjugated linoleic acids (CLA) and the sum the USFA separately. Fatty acids were analyzed and it was noted a significant variation in C16:00%, C16:00mg/g, C18:00%, C18:00mg/g, C18:1%, C18:1mg/g, CLA%, CLA mg/g, sum the of USFA% and sum the of USFA amount in mg/g ($p<0.05$) (Figures 17 and 18).

It is noted that the mean concentration of C16:00%, C16:00mg/g, C18:00% and C18:00mg/g FAs in milk fat during spring, summer, and winter has no different. Autumn samples recorded difference to spring and summer for C16:00% FAs, but no difference to winter samples, while C16:00mg/g and C18:00mg/g FAs from autumn was different to summer but not to spring and winter (Figure 18). The

mean concentration of C18:00% during autumn recorded difference to spring, summer and winter. A seasonal variation was found in the level C18:1% and C18:1mg/g in milk FAs, when milk FAs are analysed ($P<0.05$).

The level of C18:1% during winter was different from the other seasons while spring, summer, and autumn were alike. C18:1mg/g during winter was different to spring and autumn but alike to summer as in Figures 17 and 18. The mean concentration of CLA% winter was found to be different to spring, summer and autumn, while average CLA mg/g for autumn and summer was found to be different from spring and winter but like each other.

The mean for the sum of USFA% winter samples recorded difference to summer, spring, and autumn, while for the sum USFA mg/g winter samples were different to spring, and autumn but like summer.

Spring samples recorded its highest mean concentration of C16:00% of 35.79% and the lowest mean concentration of C18:1 mg/g of 64.79mg/g, while C18:00%, C18:00mg/g, C18:1% and C18:1mg/g recorded the maximum mean concentration in winter 11.128%, 39.50mg/g, 24.83% and 88.9mg/g individually as recorded in Table 14 and 15. Autumn recorded the lowest for C16:00%, C18:00% and C18:00mg/g of 32.33%, 8.58% and 27.88mg/g respectively. 20.31% was recorded as the lowest level of C18:1% in summer (Tables 13 and 14). In the summer the mean concentration of CLA mg/g was the highest 3.51mg/g, while in winter recorded the lowest for both CLA mg/g and CLA % 2.411mg/g and 0.63% respectively. The concentration of CLA % was higher in autumn at 1.01%. Winter sample showed the higher mean concentration of the sum of USFA% and sum USFA mg/g of 31.60% and 117.4mg/g respectively, while in autumn recorded the lowest mean level of the amount of USFA% of 27.21%. The lowest mean concentration of the sum of USFA mg/g of 90.74mg/g was in spring.

The same samples were analysed using one-way ANOVA for its palmitic acid (C16:00), stearic acid (C18:00), oleic acid (C18:1), conjugated linoleic acids (CLA) and the sum USFA percentages and the amount of USFA separately by changing the factor to month. During the analysis, it was evident monthly variations ($p<0.05$) to the samples from the same season.

The results of the analysis showed the level of C16:00% and C18:00%, in winter (July, August, and September) samples were significant difference between its months, while in spring (October, November, and December), October samples were different from November and December for C16:00% level while no difference for C18:00% level. In summer (January, February, and March) samples, March showed a significant difference from the other months of the same season for its C16:00% level but no difference for C18:00% level, while in autumn (April, May, and June) all samples recorded no significant difference within its months for both C16:00% and C18:00% level. No variance in the level of C16:00mg/g ($p>0.05$). The level of C18:00mg/g showed variation ($p<0.05$).

In winter (July, August, and September), July samples recorded a difference to August and September.

When the concentration of CLA% and CLA mg/g were analyzed, it was absorbed in winter (July, August and September) July samples showed significant difference when compared to August and September for the concentration of CLA% and CLA mg/g, while no difference for the months of the remaining seasons for the concentration of CLA%.

Table 14: Seasonal variation of fatty acid percentage (FA%) of whole raw milk collected over the period March 2017 to February 2018 (Results are mean ± Standard deviation). A=autumn, SP=spring, S=summer and W=winter

FA %	Spring	Summer	Autumn	Winter	Seasonal Variation
C16:0 %	35.8 ± 1.8	35.3 ± 1.8	32.3± 0.3	33.6± 4.7	SP>A
C18:0 %	10.2± 0.4	9.9 ± 0.3	8.6 ± 0.9	11.1± 2.3	W>A &SP>A
C18:1 %	20.4 ± 2.2	20.3± 1.2	21.4 ± 0.8	24.8± 4.9	W>SP
USFA %	27.8± 2.1	28.6± 0.6	27.2 ± 0.7	31.6± 5.3	W>SP, A
CLA%	0.9± 0.1	1.0 ± 0.1	1.0± 0.1	0.6± 0.2	A>SP, W&S>W, SP

In spring (October, November, and December), the sample shows no different for CLA mg/g level. In Summer (January, February, and March), March samples showed a significant difference from the other months of the same season for its CLA mg/g level, while in Autumn (April, May, and June) June samples recorded difference from other months of the same season for CLA mg/g level.

Table 15: Seasonal fatty acid (FA mg/g) of whole raw milk collected over the period March 2017 to February 2018 (Results are mean ± Standard deviation). A=autumn, SP=spring, S=summer and W=winter

FA (mg/g)	Spring	Summer	Autumn	Winter	Seasonal Variation
C16:0(mg/g)	111.7±11.5	130.5±20.7	104.7±20.4	116.5±44.2	NS
C18:0(mg/g)	32.2±5.4	36.5±4.8	27.9±6.8	39.5±16.1	W>A
C18:1(mg/g)	64.8±15.4	74.6±7.6	69.3±14.8	88.9±35.6	W>SP
USFA (mg/g)	91±19	106.3±12.4	93.7±18.6	117.4±44.7	NS
CLA (mg/g)	2.6±0.5	3.5±0.4	3.2±0.3	2.4±0.9	S>SP, W &A>W

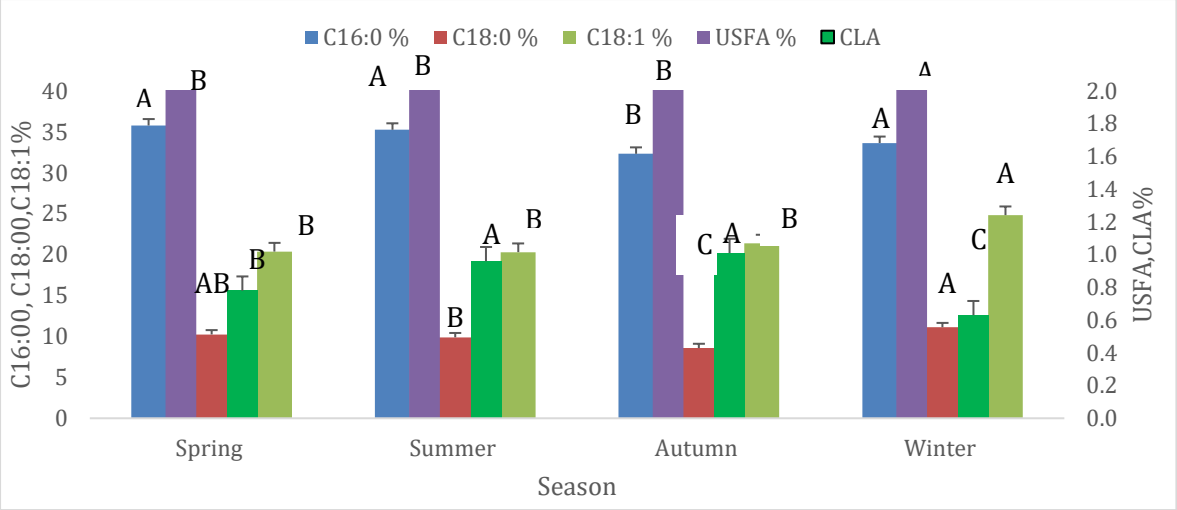

Figure 17: Seasonal variation fatty acid percentage (FA%) whole raw milk collected over the period March 2017 to February 2018 (Results are mean and grouping information). USFA% and CLA% are presented in the secondary axis. Means that do not share a letter are significantly different.

When the mean for the sum of USFA% and the sum USFA amount in mg/g were analyzed, it was absorbed in winter (July, August and September) July samples showed significant difference when compared to August and September for the concentration of the sum of USFA%, while sum USFA amount in mg/g showed difference in all the months. In spring (October, November, and December), summer (January, February, and March), and autumn (April, May, and June) recorded no difference in the mean for the sum of USFA%. The amount USFA mg/g in spring (October, November, and December), December sample was different from the other two months. July samples recorded higher mean value for C16:00% of 39.72%, while the lower level of CLA%, CLA mg/g, the sum of USFA mg/g and the sum of USFA% of 0.43%,1.61mg/g and 76.80mg/g and 25.10% respectively. The lowest; level of C16:00 was recorded in August 29.23% while the highest level of C18:00% and the sum of USFA% of 13.41% and 37.14% each were recorded to the same month.

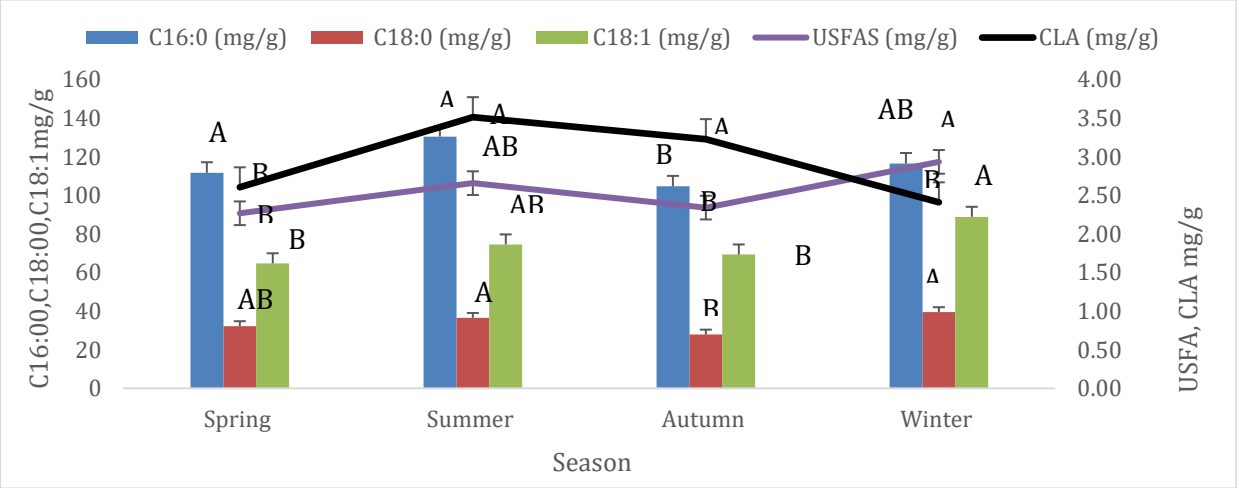

Figure 18: Seasonal variation of fatty acid (FA mg/g) whole raw milk collected over the period March 2017 to February 2018 (Results are mean and grouping information). USFA and CLA are presented by line. Means that do not share a letter are significantly different.

Table 16: Monthly difference fatty acid percentage (FA%) whole raw milk collected over the period March 2017 to February 2018 (Results are mean ± Standard deviation).

Months	C16:0	C18:0	C18:1	USFA	CLA
October	33.91 ± 2.00	10.65 ± 0.41	22.54 ± 2.70	29.87 ± 2.87	0.79 ±0.10
November	36.41 ± 0.53	10.01 ± 0.29	19.17 ± 0.64	26.92 ± 0.29	0.78 ±0.02
December	37.06 ± 0.43	10.10 ± 0.27	19.45 ± 1.28	26.78 ± 0.25	0.78 ±0.04
January	36.03 ± 0.53	10.00 ± 0.37	19.69 ± 0.53	28.56 ± 0.57	0.88 ±0.04
February	36.17 ± 0.43	9.94 ± 0.34	19.96 ± 0.78	28.82 ± 0.69	0.92 ±0.09
March	31.92 ± 0.02	9.67 ± 0.02	22.25 ± 0.00	28.12 ± 0.00	1.23 ±0.00
April	32.28 ± 0.27	9.24 ± 0.18	21.56 ± .058	27.32 ± 0.58	1.09 ±0.04
May	32.00 ± 0.11	8.47 ± 0.67	21.56 ± 0.14	27.52 ± 0.16	1.10 ±0.07
June	32.71 ± 0.05	8.03 ± 1.28	21.01 ± 1.39	26.79 ± 1.00	0.86 ±0.05
July	39.72 ± 1.53	8.43 ± 1.34	18.93 ± 0.70	25.10 ± 1.81	0.42 ±0.29
August	29.24 ± 0.88	13.41 ± 0.97	30.07 ± 1.69	37.14 ± 1.60	0.74 ±0.01
September	32.00 ± 0.30	11.54 ± 0.08	25.49 ± 0.57	32.56 ± 0.66	0.72 ±0.01

Table 17: Monthly difference fatty acid (FA mg/g) whole raw milk collected over the period March 2017 to February 2018 (Results are mean ± Standard deviation).

Months	C16:0	C18:0	C18:1	USFA	CLA
October	118.87±6.40	37.62 ± 5.48	80.13± 18.08	109.21± 21.98	2.81± 0.64
November	103.86±16.65	28.44±3.72	55.62±7.61	79.00±11.19	2.22±0.29
December	112.46±4.14	30.68±2.13	58.61±3.25	84.03±4.45	2.79±0.29
January	128.74±9.49	35.64 ± 1.59	70.26±3.38	101.94±5.17	3.13±0.13
February	148.84±3.35	40.92± 2.69	82.20±5.68	118.67±6.44	3.78±0.48
March	97.45±1.01	29.49± 0.33	67.92±0.67	90.57±0.86	3.74±0.04
April	95.85±2.77	27.43± 0.20	63.97±0.63	85.79±0.55	3.23±0.05
May	97.26±5.36	25.65± 0.75	65.50±2.96	88.95± 4.44	3.31±0.06
June	120.87±30.99	30.57± 12.27	78.59± 24.90	106.26±30.46	3.13±0.62
July	112.33±73.59	22.68±14.81	53.11±34.54	76.76± 50.46	1.61±1.09
August	102.51±2.24	47.06±3.93	105.49±7.12	133.75± 7.16	2.58±0.02
September	134.75±31.96	48.77±12.59	108.03±28.80	141.63±37.48	3.04±0.78

The concentration of C18:00% was lowest in June at 8.03%. The highest concentration of C18:00mg/g and the sum of USFA mg/g was noted in September 48.77mg/g and 141.60mg/g respectively, while the lowest level of C18:00mg/g of 25.65mg/g was in May. The concentration of CLA% was higher in March 1.23%, while CLA mg/g was higher on February 3.78mg/g as shown in Table 14.

4.2.10-Mineral composition

The investigation was done for each mineral separately. During the study, it was noted a significant variation ($p<0.05$) on the level of Ca, K, Mg, Na, P, and S when the samples on a seasonal basis as in Figure 13. The results of the analysis showed the following.

The average concentration of Ca for milk from autumn, winter, and summer are different from each other, while summer is similar to spring. It also noted that winter is identical to spring as shown in

Figure 13. The higher average Ca concentration was in autumn (1715.8mg/L), while a lower average level of Ca was in summer (1196.2mg/L) (Table 17).

Table 18: Seasonal minerals level whole raw milk in mg/L collected over the period March 2017 to February 2018 (Results are mean ± Standard deviation). A=autumn, SP=spring, S=summer and W=winter

Minerals	Spring	Summer	Autumn	Winter	Seasonal Variation
Ca	1253±119	1196 ±205	1716±172	1413 ±110	A>W, SP, S & W>S
K	1404± 26	1352 ± 234	984 ± 39	1270 ± 108	W>SP, SU, A
Mg	104 ± 10	112 ± 21	159 ± 13	121 ± 11	A>W, SP, S & SP>W
Na	329 ± 5	339 ± 22	460 ± 45	414 ± 77	A>S, SP &W>S, SP
P	1013 ± 61	848± 88	1032 ± 62	1096 ± 49	W>SP, S&SP>S
S	320± 7	306 ± 67	460 ± 31	354 ± 39	A>W, SP, S & W>S

The average concentration of K in milk from spring, summer, and winter recorded no significant differences. Autumn samples were found significantly different (Figure 19). The average K level of milk was higher in spring (1404.3 mg/L) and lower in autumn (984.3 mg/L) (Table 18).

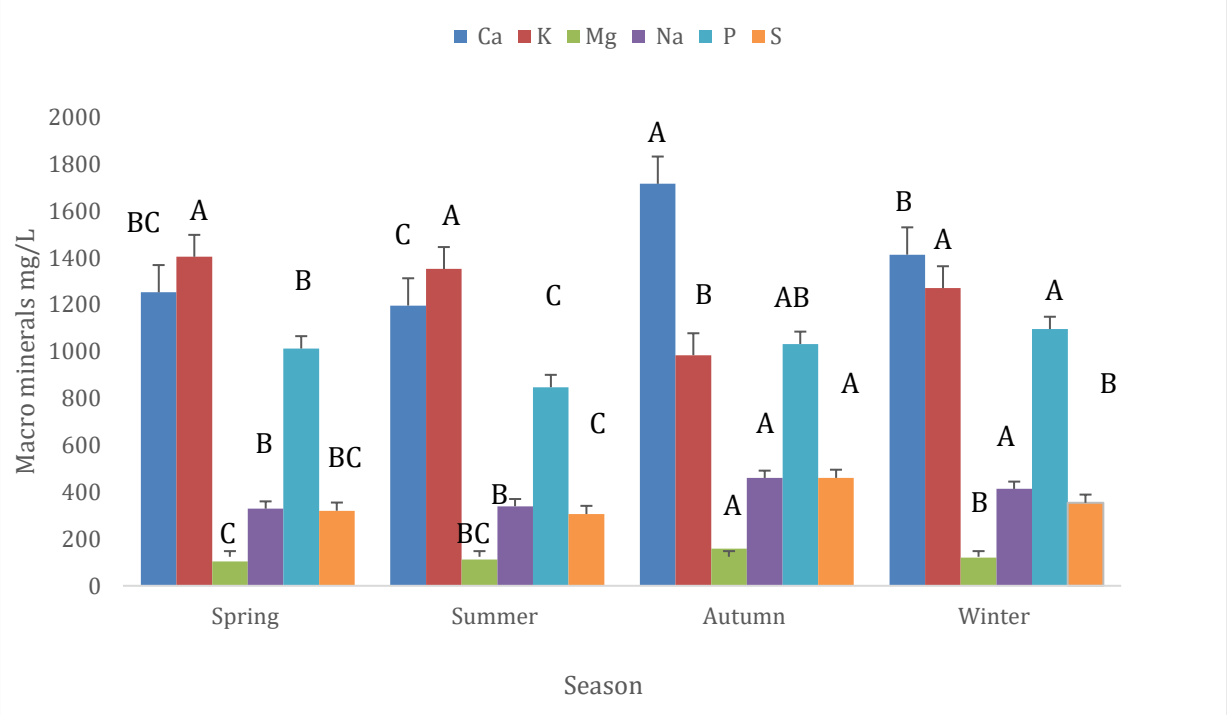

Figure 19: Seasonal minerals level in mg/L of whole raw milk collected over the period March 2017 to February 2018 (Results are mean and grouping information). Means that do not share a letter are significantly different.

The average Mg concentration of milk from summer and spring were not different, the same between winter and summer too. Spring, winter, and autumn samples were different from each other (Figure 19). The average Mg level of milk was higher in Autumn (158.54 mg/L), and lower in Spring (104.2mg/L) (Table 18).

Table 19: Monthly minerals level whole raw milk in mg/L collected over the period March 2017 to February 2018 (Results are mean ± Standard deviation).

Months	Ca	K	Mg	Na	P	S
October	1157±149	1376±26	96±12	326±7	980±78	314±6
November	1345±74	1416±6	111±4	331±3	1065±38	321±5
December	1256±32	1422±13	106±2	330±3	993±17	326±5
January	1126±15	1400±203	101±1	322±13	846±60	286±53
February	1076±69	1467±170	103±2	341±19	780±15	274±40
March	1576±2.91	1023±21	152±7	370±5	987±37	411±10
April	1588±170	997±37	146±13	408±9	987±47	431±15
May	1816±98	998±29	166±8	464±18	1062±52	486±27
June	1743±183	958±45	163±11	509±19	1047±72	465±22
July	1501±42	1129±26	133±2	516±6	1090±32	403±7
August	1409±144	1322±31	117±11	380±16	1127±69	343±18
September	1330±43	1361±18	112±3	345±7	1070±32	317±6

The average concentration of Na in milk from summer and spring recorded no difference, the same in autumn and winter too. Spring and summer samples were significantly different from winter and autumn samples (Figure 19). The average Na concentration in milk was higher in autumn (460.3 mg/L), and lower in spring (329.20 mg/L) (Table 18). The average P concentration in milk during spring and autumn recorded no difference to each other and the same for samples from autumn and winter. Spring, autumn and winter samples were significantly different to summer (Figure 19). The average P concentration in milk was higher in winter (1095.7 mg/L), and lower in summer (847.6 mg/L) (Table 19).

The average sulphur level of milk from spring and summer was found to be similar. Similarity also recorded between spring and winter. Summer, autumn, and winter were significantly different to each (Figure 19). The average S concentration in milk was higher in autumn (460.65 mg/L) and lower in summer (306.3 mg/L) (Table 18).

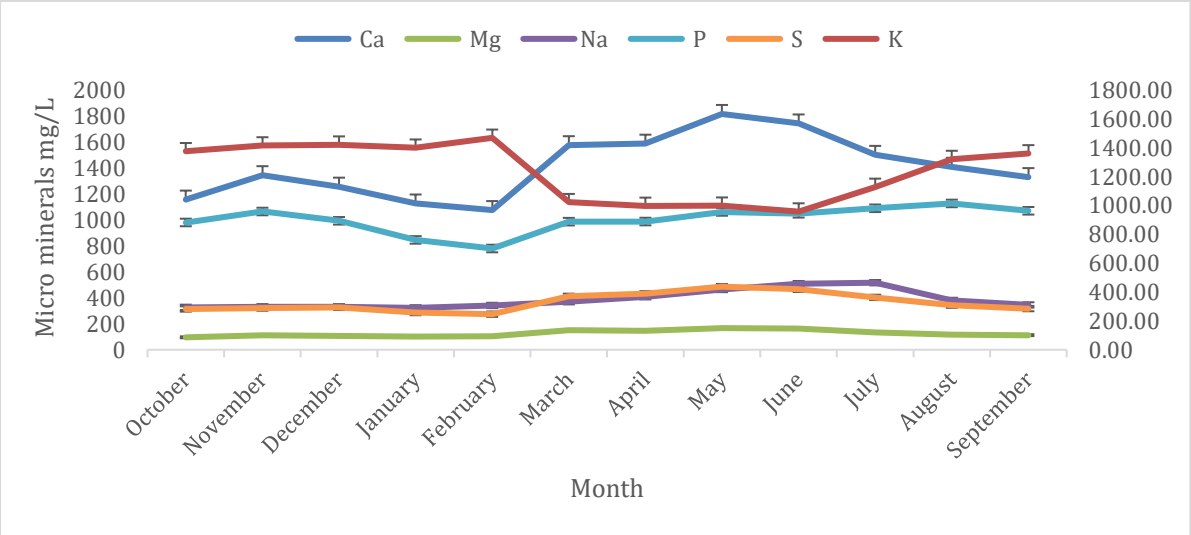

Figure 20: Monthly difference of minerals composition of fresh whole milk over the period of March 2017 to February 2018 monthly.

4.3-Physical properties of raw milk

4.3.1-Particle size distribution (PSD)

Fresh whole milk samples collected from Lincoln University dairy farms in Canterbury during different seasons (March 2017 till February 2018) were analysed using one-way ANOVA, to investigate if it is seasonal and monthly variation for its surface area moment mean and-D (3,2) and Volume moment mean-D (4,3). The investigation recorded a significant difference ($p<0.05$) in its D (3, 2) and D (4, 3) between different seasons as shown in figure 16. Winter was found to be different form spring, summer and autumn samples for its D-(4,3), while summer is different to samples from spring, winter, and autumn for its D-(3,2).

Spring, summer and autumn samples showed no difference to each other for D-(4, 3), while spring, winter, and autumn showed no difference for its D (3, 2). Winter sample recorded the highest for both D-(3,2) and D-(4,3) 0.26±0.04µm and 3.13±0.29µm, while autumn noted the lowest D-(3,2) and D-(4,3) of 0.24±0.03µm and 2.44±0.38µm respectively (table 8). When the same analysis was carried by changing the factor from seasons to months, there was no significant variation ($p=0.05$) within months of the season (Figure 21).

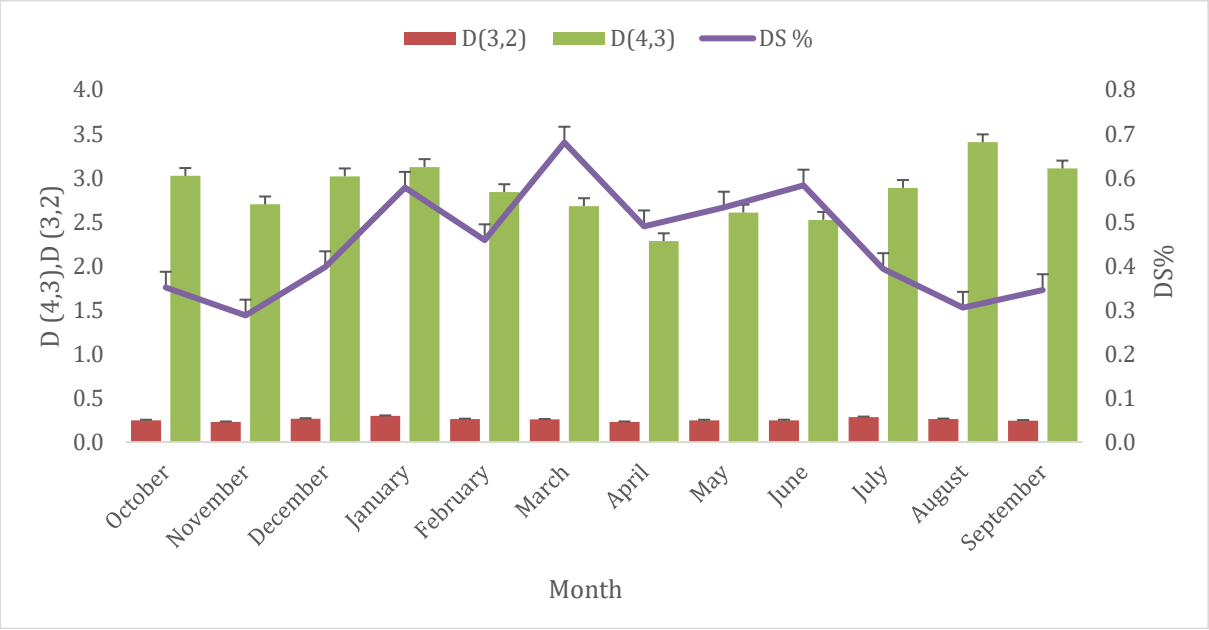

Figure 21: Monthly variation of particle size distribution (PSD µm) (D [3, 2] - Surface weighted mean and D [4, 3] - Volume weighted mean) and dry sedimentation percentage (DS%) in fresh whole milk collected over the period March 2017 to February 2018 (Results are mean and Standard deviation). DS% is presented in secondary axis. Means that do not share a letter are significantly different.

4.3.2-Ethanol stability (ES)

Fresh whole milk was investigated if there was a seasonal or monthly variation in its heat stability using the ethanol test. The investigation recorded a significant difference ($p<0.05$) in its ES% between different seasons as (Figure 22). Autumn sample recorded the highest ES value

45.57%±10.66 while summer noted the lowest ES% of 20.28%±7.49 (table 8). Spring and summer were found to be like each other but different to autumn and winter (Figure 22). When the same analysis is done by changing the factor from seasons to months, there was a significant difference ($p<0.05$) within months of the season (Table 10). May and June samples were different from January, February, September, October, November, and December samples.

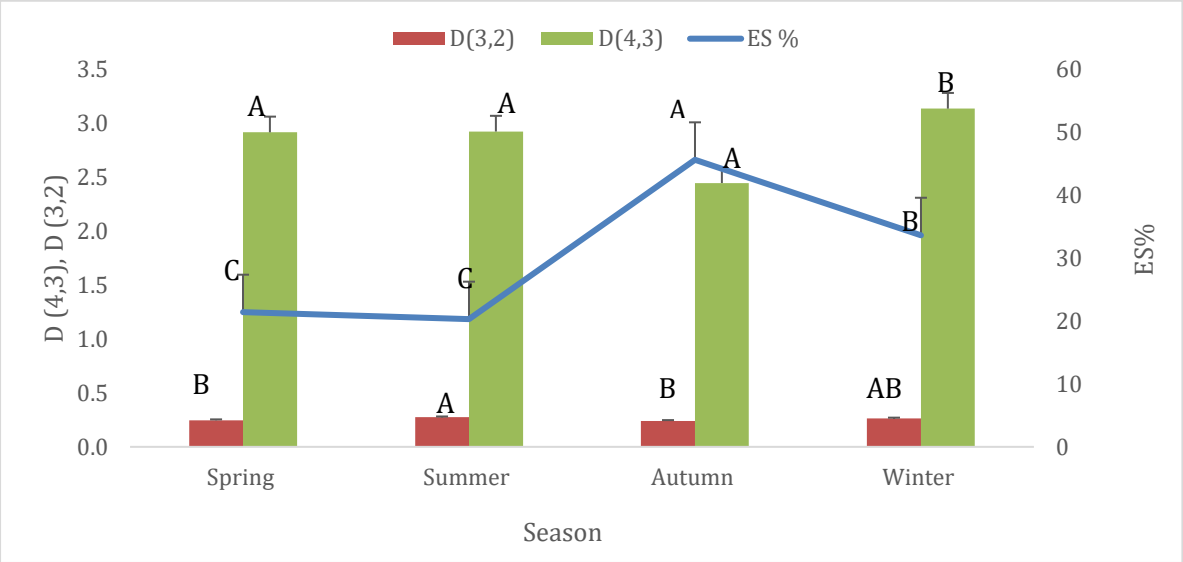

Figure 22: Seasonal variation of ethanol stability (ES%) and particle size distribution (PSD µm) (D [3, 2] Surface weighted mean and D [4, 3] - Volume weighted mean) of fresh raw milk collected over the period March 2017 to February 2018 (Results are mean standard deviation and grouping). ES% is presented in the secondary axis. Means that do not share a letter are significantly different.

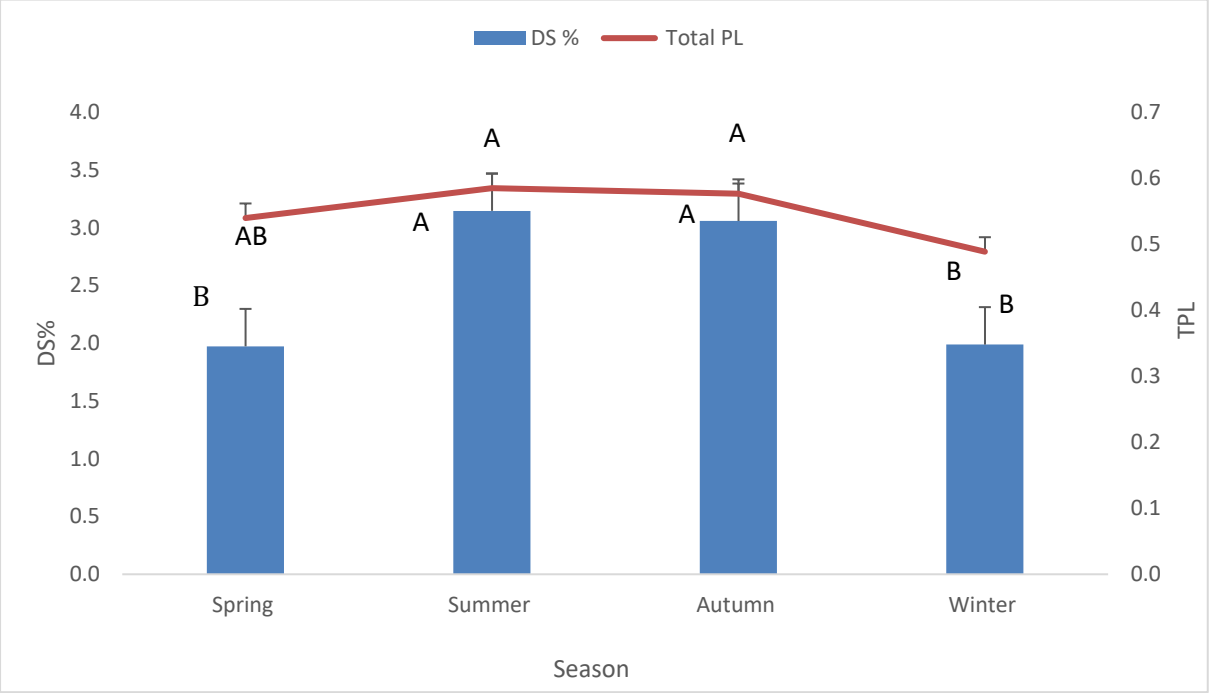

Figure 23: Seasonal variation of dry sedimentation rate (DS%) and total phospholipids (TPL µL/M) in fresh whole milk collected over the period March 2017 to February 2018 (Results are mean and Standard deviation and grouping information). TPL is presented in the secondary axis. Means that do not share a letter are significantly different.

4.3.3-Dry sedimentation (DS %)

The investigation recorded a significant variation ($p<0.05$) in the sedimentation rate between different seasons (Figure 23). Spring and winter samples were found to be similar to each other but were different from summer and autumn samples. Summer and autumn recorded no difference. The highest rate of sedimentation was recorded in summer 0.55±0.12%, while the lowest sedimentation rate was in winter 0.21±0.21% (table 8). When the same analysis was done by changing the factor from seasons to months, there was no significant difference ($p>0.05$) within months of the season (Table 10).

4.4-Interrelationship between raw milk compositions

The significant correlation between milk components was evident (Tables 20 and 21). The fat content for the raw milk was found to have strong positive correlation to protein, minerals (Na, Mg, S, and Ca), and ethanol stability (Table 19).

Table 20: Correlation coefficient and p=Value for raw milk composition collected over the period from March 2017 to February 2018

Components	CC	pV	Components	CC	pV
Fat/Protein	0.983	0.000	TS/TPL	0.487	0.001
Fat/TS	0.426	0.006	TS/D (4,3)	-0.435	0.003
Fat/C18:0 (mg/g)	-0.425	0.003	TS/K	-0.360	0.014
Fat/USFA%	0.329	0.026	TS/Na	0.324	0.028
Fat/TPL	0.384	0.010	TS/Mg	0.295	0.046
Fat/ES	0.702	0.000	TS/S	0.300	0.043
Fat/D (4,3)	-0.5	0.001	Ca2+/DS	0.322	0.029
Fat/Ca	0.818	0.000	Ca2+/ES	0.443	0.003
Fat/K	-0.867	0.000	Ca2+/CLA%	0.570	0.000
Fat/Mg	0.898	0.000	Ca2+/CLA mg/g	0.344	0.019
Fat/Na	0.891	0.000	Ca2+/Ca	0.406	0.005
Fat/S	0.884	0.000	Ca2+/K	-0.458	0.001
Protein/TS	0.402	0.006	Ca2+/Mg	0.424	0.003
Protein/Ca ion	0.296	0.046	Ca2+/S	0.364	0.013
Protein/BC	0.42	0.004	pH/P	0.401	0.006
Protein/DS	0.338	0.022	BC/DS	0.366	0.012
Protein/USFA%	-0.382	0.009	BC//D (4,3)	-0.392	0.009
Protein/TPL	0.481	0.001	BC/USFA%	-0.357	0.015
Protein/C18:0 (mg/g)	-0.495	0.000	BC/K	-0.387	0.008
Protein/ES	0.664	0.000	BC/Na	0.324	0.028
Protein/D (4,3)	-0.506	0.000	BC/S	0.343	0.020
Protein/Ca	0.804	0.000	BC/TPL	0.338	0.025
Protein/K	-0.849	0.000	P/S	0.512	0.000
Protein/Mg	0.889	0.000	S/TPL	0.303	0.046
Protein/Na	0.847	0.000	Na/P	0.427	0.003
Protein/S	0.869	0.000	K/S	-0.833	0.000

It was also positively correlated to the total solids and negatively correlated to C18:00 mg/g and D (4,3). The protein concentration of raw milk was strongly positively correlated to minerals (Na, Mg, S,

and Ca), and ethanol stability (Table 19). It was also correlated to TS, BC, TPL, while negatively correlated C18:00 mg/g and D (4,3).

The concentration of Ca in raw milk was found to be strongly positively correlated to P, Na, and S, while negatively correlated to K. Potassium was strongly negatively correlated to Mg, Na, and S, and weakly negatively correlated to P. Sodium was positively correlated to P and S (Tables 19 and 20). It was also observed that P was correlated to S. The correlation between S and TPL was also found to be weak (Table 19).

Ca2+ concentration was positively correlated to DS, ES, CLA and minerals (Ca, Mg, and S) but negatively correlated to K (Table 19).

Table 21: Correlation coefficient and p=Value for raw milk composition collected over the period from March 2017 to February 2018.

Components	CC	pV	Componets	CC	pV
C16:0mg/g/C18:0mg/g	0.697	0.000	USFA%/TPL	-0.397	0.008
C16:0(mg/g)/USFA%	0.748	0.000	USFA mg/g/CLAmg/g	0.580	0.000
C16:0(mg/g)/CLA mg/g	0.565	0.000	USFA%/TPL	-0.433	0.003
C16:0(mg/g)/Ca	-0.372	0.011	CLA%/CLA mg/g	0.760	0.000
C16:0(mg/g)/P	-0.351	0.017	CLA%/K	-0.306	0.038
C16:0(mg/g)/S	-0.351	0.017	CLA%/Mg	0.353	0.016
C18:0mg/g/USFA%	0.789	0.000	CLA%/TPL	0.327	0.030
C18:0mg/g/USFA mg/g	0.962	0.000	CLA%/whey	-0.352	0.019
C18:0mg/g/CLA mg/g	0.506	0.000	CLA%/Casein	-0.302	0.046
C18:0mg/g/Ca	-0.413	0.004	Ca/K	-0.809	0.000
C18:0mg/g/K	0.384	0.008	Ca/Mg	0.959	0.000
C18:0mg/g/Mg	-0.409	0.005	Ca/Na	0.751	0.000
C18:0mg/g/Na	-0.398	0.006	Ca/P	0.641	0.000
C18:0mg/g/S	-0.471	0.001	Ca/S	0.907	0.000
C18:0mg/g/TPL	-0.471	0.001	K/Mg	-0.867	0.000
USFA%/USFA mg/g	0.738	0.000	K/Na	-0.741	0.000
USFA%/Na	-0.317	0.032	K/P	-0.317	0.032
USFA%/S	-0.303	0.041	Na/S	0.808	0.000
DS/D (3,2)	0.365	0.015	ES/Ca	0.692	0.000
DS/CLA%	0.372	0.011	ES/K	-0.675	0.000
DS/K	-0.301	0.042	ES/Mg	0.716	0.000
DS/Mg	0.336	0.022	ES/Na	0.671	0.000
DS/S	0.309	0.037	ES/S	0.705	0.000
DS/TPL	0.433	0.003	ES/P	0.381	0.013
D (3,2)/D (4,3)	0.686	0.000	D (4,3)/CLA%	-0.407	0.006
D (4,3)/C16:00mg/g	0.415	0.005	D (4,3)/Ca	-0.392	0.009
D (4,3)/USFA%	0.446	0.002	D (4,3)/K	0.432	0.003
D (4,3)/USFA mg/g	0.299	0.049	D (4,3)/Mg	-0.500	0.001

BC was positively correlated to DS, Na, S and TPL but negatively correlated to K. The rate of DS was positively correlated to the D (3,2) CLA%, Mg, S while negatively correlated to K. ES was strongly positively correlated to Ca, Mg, Na, and S and weakly correlated to P. ES was strongly negatively correlated to K (Table 19). When FA was analysed, a strong positive correlation of

C16:0mg/g/C18:0mg/g, C16:0(mg/g)/USFA%, C18:0mg/g/USFA%, C18:0mg/g/USFA mg/g, and USFA%/USFA mg/g, CLA%/CLA mg/g were observed. D (4,3) was strongly positively correlated to D (3,2), while weak significantly correlated to C16:0mg/g, USFA% and K. It is also weakly negatively but significantly correlated to CLA%, Ca and Mg.

4.5- Principle Component Analysis (PCA)

Principle component analysis (PCA) was carried out on the data raw milk collected during April 2017 and February 2018. The data set consisted of 23 samples and 24 variables. The PCA similarity map defined by principal components PC 1 and PC 2 showed a discrimination of samples according to the different seasons. In the PCA, the similarity map is defined by PC1 and PC2. Milk samples were separated according to PC1 with a variation of 37.79% (Figures 17 and 18).

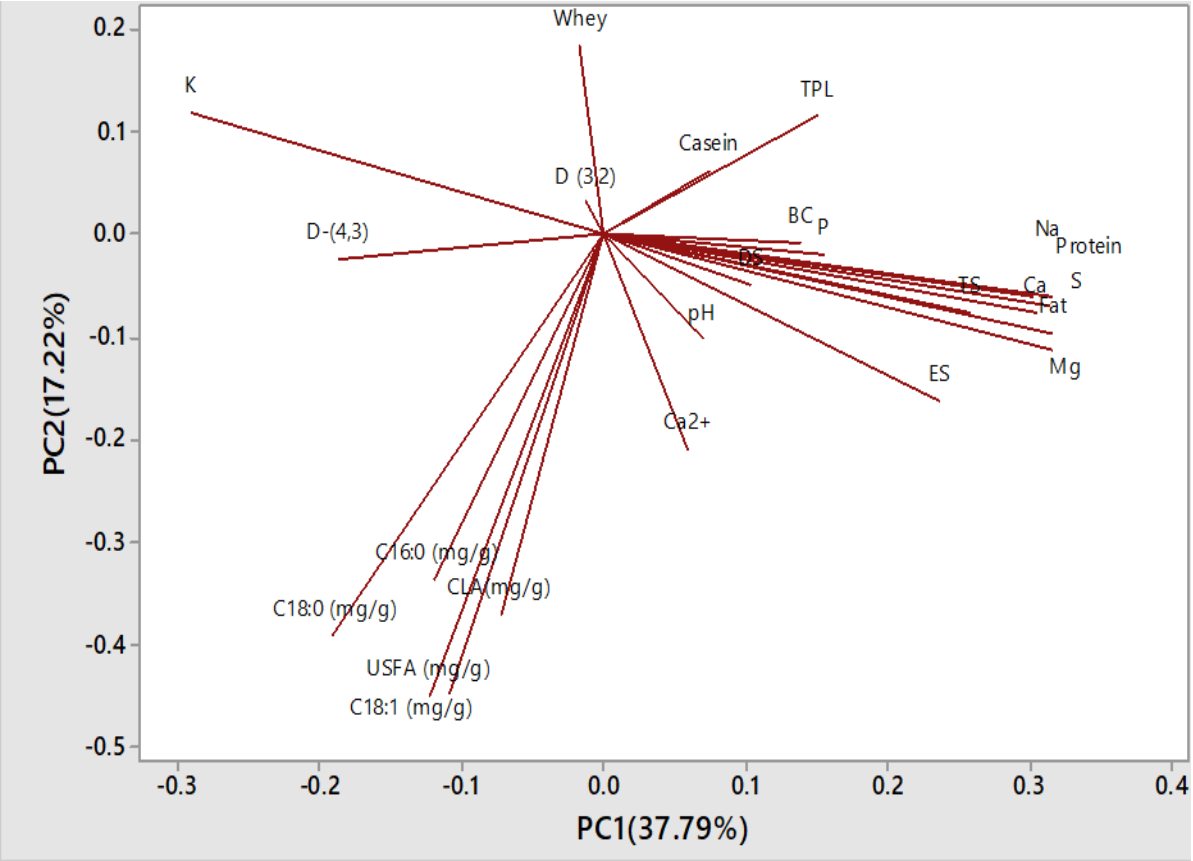

Figure 24: the effect of seasonal variation on raw milk compositions according principle component analysis (PCA) similarity map, determined by principle component PC1(37.79%) and PC2 (17.22%)

Autumn milk showed discrimination from milk of the other seasons which showed a variation of 37.79% (Figure 17). Milk from summer, spring and winter showed small variations. The PCA in Figure 18 clearly showed the effect of the seasonal variations in milk compositions. All samples from autumn and most of spring, summer and few from winter were in the positive part of the similarity map, whereas some from spring, summer and winter samples were on the negative part (Figure 17).Raw milk in autumn and winter were characterised by higher fat, protein, ES, RCT, TS, Ca, Mg,

Na, S, and Ca^{++}, while higher TPL was found in the winter milk. Spring, summer and winter samples were characterised by higher FA amount. Similarity map determined by principle component PC1(37.79%) and PC2 (17.22%).

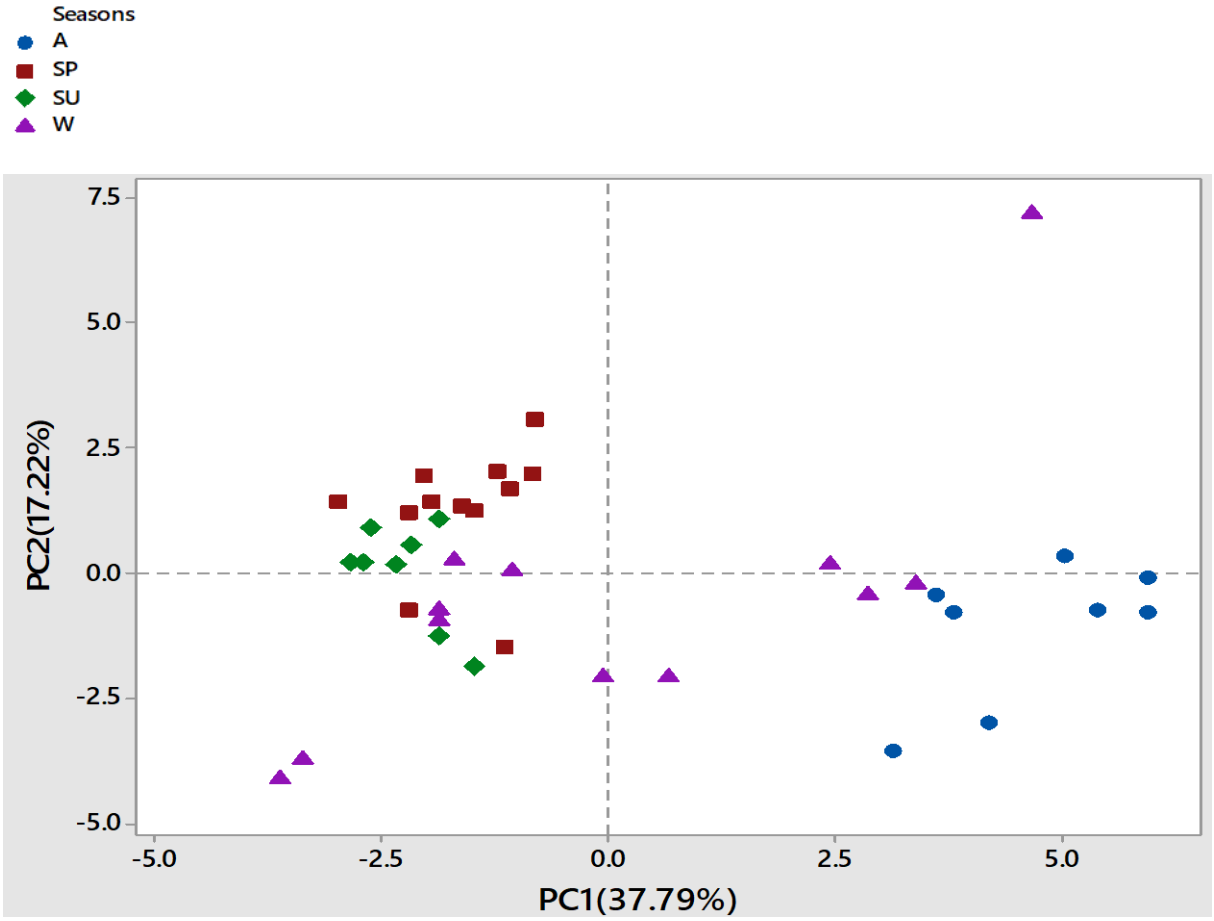

Figure 25: Seasonal variation on raw milk according principle component analysis (PCA). A= autumn, SP= spring, SU= summer and W=winter

4.6- Discussion

In this study, raw milk investigation carried for its seasonal variation in its physical properties and chemical composition. Seasonal variation in milk physical properties and chemical composition is described. In the current experiment, a particular constituent of raw milk from cows at different seasons was compared throughout the year, while holding another variable constant.

This study showed a seasonal variation in milk protein and fat concentrations which agrees with the finding of (Walker, Williams, Doyle, & Dunshea., 2007). During the study, it was evident that raw milk from autumn was significantly different ($p<0.05$) in its protein and fat concentrations. In autumn the fat and protein concentrations were high which is generally in line with the findings from DairyCo. (2013). The fat and protein content declined during spring and summer, recording its lowest in spring. In spring cows produce the maximum milk production, while the fat and protein content are on its lowest level. The protein and fat concentrations tend to be higher at the beginning and the

end of the lactation period when compared with the middle period (Bansal et al., 2009). The fat concentration from summer and spring and the protein concentrations from summer and winter recorded no significant variations, similar to the protein concentration in summer and spring (Figure 4). The decline in the fat concentration of milk from autumn to summer and spring is more likely to be due to the change in temperature. During summer, the temperature increases and the mean annual maximum temperature in Canterbury can reach up to 32℃. When the environmental temperature increases, the synthesis of FAs is decreased, especially that FAs that are not derived directly from blood and the feed intake also decreases both the quantity and quality of milk (Butler et al., 2008). The result observed challenges the findings of A. Yasmin, Huma, Sadiq Butt, Zahoor, and Yasin (2012) and Rehman, Khan, and Mirza. (2014), who concluded that the fat concentration reaches a minimum in summer and a maximum in winter. The decline of protein may be the result of high temperatures and the progress of the lactation stage (Sevi et al., 2001). Amino acids (AA) are the building blocks of protein. During spring and summer, the increased temperature decreases the milk protein concentration. During these periods, the cow may be using more amino acids for energy production to meet additional requirements. Therefore, the available AA may be low for milk protein production (Cowley et al., 2015).

The highest total solids percentage (TS %) was recorded in summer, but there was no significant seasonal variation in TS%. In a study conducted by Lindmark-Månsson, Fondén, and Pettersson (2003), the result of their research was contradictory to my study. They found it to be higher in autumn than in summer.

This results from this investigation recorded the difference in pH value between winter and summer (Figure 6). The highest pH was recorded in winter (6.72) and lowest in summer (6.67) (Table 8). The pH finding from this study is different to the observations of (Chen, Lewis, & Grandison., 2014), who observed a higher pH in spring than in summer and autumn.

The buffering capacity (BC) of fresh whole milk recorded a significant variation ($p<0.05$) between seasons (Figure 8) which is not in agreement with the authors mentioned in the above paragraph. Autumn milk was significantly different to winter and spring but not to summer milk (Figure 8).

My study recorded a significant variation ($p<0.05$) in the TPL between different seasons, with values in summer highest and in winter lowest.

The study recorded a significant variation ($p<0.05$) in the total whey protein and a-casein, with winter values highest while the lowest concentration of whey and casein were in autumn (Table 12).

The Ca^{++}concentration ranged between 74mg/L to 122.5mg/L with significant seasonal differences ($p<0.05$), which is contrary to the findings of Chen et al. (2014). The highest Ca^{++} concentration was noted in autumn (115.50mg/L), while the lowest was in spring (92.33mg/L) (Table 8). Values recorded in autumn was different to that in spring and winter but no different to summer

recordings. No difference was recorded between samples collected in spring, summer, and winter (Figure 7).

Fatty acid composition in milk can vary broadly to numerous factors, mainly seasonal environmental factors (Glover et al., 2012). In this investigation, a seasonal effect on FAs composition was observed. In general, the concentrations of individual FAs were similar in spring, autumn, and winter for C16:00 and C18:00 but different in summer ($p<0.05$), while for C18:1 and USFA were similar in spring, summer and autumn but different in winter ($p<0.05$). CLA concentrations were similar between winter and spring, and between summer and autumn but winter and spring were different from summer and autumn ($p<0.05$) (figures 10 and 11). The CLA concentration differed significantly between the seasons ($P<0.05$). In summer, the mean concentration of CLA mg/g was highest (3.51mg/g) but lowest in winter (2.41mg/g). This result was found to be similar to a study done by (Samková & Węglarz, 2012). This study reported that the CLA concentration in the summer was higher than in winter. The concentration of total UNSFA in winter samples were different to those in spring, and autumn but similar to summer. The highest total UNSFA were in winter (117.4mg/g) but declined to its lowest in spring (90.74mg/g). This finding is similar to a study reported by (Thomson & Poel, 2000).

The average concentrations of Ca, K, Mg, Na, P, Zn, and S showed a significant variation ($p<0.05$) (Figure 12). The Ca, Mg, K and S from autumn were different to those from spring, summer, and winter. The Ca, and S from winter samples was different to those in summer while for Mg, winter samples were different to those taken in spring. The Ca, Mg, Na, P, and S concentrations of milk from autumn recoded the highest concentration of 1716,158.54,460,1032 and 461 mg/L respectively, while for K maximum recorded was 1404 mg/L in spring. The finding regarding to the average concentration of Ca and Mg, is different to the study reported by (Bates & Prentice, 1996; Debry, 2001; Mapekula, Chimonyo, Mapiye, & Dzama, 2011), which state the average Ca and Mg was found to be at its lowest level in autumn, while the maximum was in spring. The same scholars also reported the average concentrations P reached a maximum in winter and a minimum in autumn which is different to my results. The Ca, P, and S concentrations declined to 1196, 848 and 306 mg/L respectively in summer. The minimum concentration of Mg and Na were recorded in spring 104 and 329 mg/L respectively, while minimum concentration of K was noted in autumn (984mg/L). The Na concentration was minimal in autumn and maximum in winter in the study of (Bates & Prentice, 1996) which is different to my results. The minimum K concentration was in winter contradictory to the finding of Bates and Prentice (1996), but the maximum was in spring which is in agreement with the findings of Bates and Prentice (1996).

This investigation recorded a significant variation ($p<0.05$) in the particle size D (3, 2) and D (4, 3) between different seasons (Figure 14). Samples taken in winter was different to that in spring,

summer and autumn samples for its D-(4,3), while summer samples were different to the samples from spring, winter, and autumn for its D-(3,2). Spring, summer and autumn samples showed no difference to each other for D-(4, 3), and spring, winter, and autumn showed no difference in its D (3, 2). Winter sample recorded the highest D-(3,2) and D-(4,3) 0.26µm and 3.13µm while in autumn the lowest D-(3,2) and D-(4,3) of 0.24µm and 2.44µm respectively were noted (Table 8).

ES% recorded a seasonal variation ($p<0.05$) is shown in Figure 7. Autumn samples recorded the highest ES% 46%±11 than in other seasons, while in summer lowest ES% of 20%±7 were observed (Table 8). Samples from autumn, winter, and spring samples were significantly different from each other, while spring and summer samples were not much different from each other (Figure 14). The results of ES% reported by Chavez et al. (2004) were higher than the result from my study. The average ES% were less than 45.57%. The result of my study is different to the findings of Chen et al. (2014), who found ES% from spring to be higher than in autumn.

The dry rate sedimentation recorded a significant seasonal variation ($p<0.05$) (figure 15). The values from winter and spring samples were similar to each other but different to those samples taken in summer and autumn, while summer and autumn recorded no significant differences. The highest rate of sedimentation in raw milk was recorded in summer and autumn with values of 0.55% and 0.54% respectively while the lowest sedimentation rate was noted in spring and winter raw milk 0.35% and 0.21% respectively (table 8) which are in agreement with the findings Chen et al. (2014).

Fat and protein concentrations of the raw milk were found to be strong positively correlated to each other and the minerals (Na, Mg, S, and Ca), and ES (Table 19). The correlation between ES, fat and protein of this study agrees with the findings of (Chen et al., 2014). The fat and protein were also positively correlated to total solids which agrees with the findings of Chen et al. (2014) but negatively correlated to with K, C18:00 mg/g and D (4,3). This paper found no correlation to Ca^{++} and pH, which disagrees with the findings of On-Nom, Grandison, and Lewis (2010) who concluded a weak but a significant negative correlation between pH and Ca^{++}. The Ca, Na, Mg, S and P concentration in the raw milk was strongly positively correlated to each other but negatively correlated to K (tables 19 and 20). The correlation between S and TPL was also weak (Table 19). Ca^{++} concentration was positively correlated to DS, ES, CLA and to Ca, Mg, and S, but negatively correlated with K (Table 19). BC was positively correlated to DS, Na, S and TPL but negatively correlated to K. The rate of DS was positively correlated to the D (3,2) CLA%, Mg, S but negatively correlated to K. ES was strongly positively correlated to Ca, Mg, Na, and S and weakly correlated to P. ES was also strongly negatively correlated to K (Table 19).

Among FAs, a strong positive correlation of C16:0mg/g/C18:0mg/g, C16:0(mg/g)/USFA%, C18:0mg/g/USFA%, C18:0mg/g/USFAmg/g, and USFA%/USFAmg/g, CLA%/CLAmg/g were recorded.

D (4,3) was strongly positively correlated to D (3,2) but weak and significantly correlated to C16:0mg/g, USFA% and K, CLA%, Ca and Mg.

The PCA similarity map defined by principal components PC 1 and PC 2 showed a discrimination of samples according to the different seasons. Milk samples were separated according to PC1 with a variation of 37.79% (Figures 17 and 18). Autumn milk showed discrimination from milk of other seasons with a variation of 37.79% (Figure 17). Milks from summer, spring and winter showed minor variations.

All samples collected during autumn and most of spring, summer and few from winter were localised in the positive part of the similarity map, whereas some samples from spring, summer and winter were in the negative (Figure 17).

Raw milk in autumn and winter were characterised by higher fat, protein, ES, RCT, TS, Ca, Mg, Na, S, and Ca^{++}, while higher TPL was found in winter milk. Spring, summer and winter samples were characterised by higher FAs.

Chapter 5

Processed skimmed milk analysis

5.1-Introduction

This chapter shows the result of the study on how skim milk properties affect the heat stability of processed skim milk. Skim milk samples used during the study were obtained from fresh raw milk collected from Lincoln University dairy farm between May 2017 to February 2018. All investigations were carried out under standardised conditions. General liner variation was used to investigate the variation between the seasons, the treatment and the interaction between seasons and treatment. Three litters of skimmed milk are prepared from a centrifuged fresh milk at 3000 x g for 30 min using a high-speed Beckman j2-MI centrifuge with rotary JA-10 (Backman, J2-MI, US Florida), and the fat will be removed carefully yielding fresh skimmed milk (FSM). An aliquot of FSM (220ml) will be high sheared (11,000 rpm for 10 min, HS) and heat treated (HT), while the second aliquot will be high sheared after heat treatment using a high shear mixer (Polytron, Polytron 3100D, Luzern). Processed skim milk was used to investigate milk heat stability by eliminating the impact of milk fat globules and their associated materials.

During the processing, stabiliser salts were used to improve the heat stability of skim milk. Ten mM of Di-sodium hydrogen phosphate (DSHP), tri-sodium citrate (TSC) and Sodium dihydrogen phosphate (SDHP) and di-sodium hydrogen phosphate (DSHP) as a mixture in a ratio of (2:1) are added to skim milk before processing. The investigation of pH, Ca^{++}, sedimentation rate (DS), particle size distribution, and colour measured on day 1(D1) and day 30 (D2) from the processing time. During data analysis Di-sodium hydrogen phosphate (DSHP) is referred as S1, tri-sodium citrate (TSC) noted as S2 and Sodium dihydrogen phosphate (SDHP) and di-sodium hydrogen phosphate (DSHP) noted as S3.

Properties	Milk sample	D1	D2	D1	D2	D1	D2	D1	D2
		Spring	Spring	Summer	Summer	Autumn	Autumn	Winter	Winter
pH	HS+HT C	6.62±0.02	5.91±0.36	6.55±0.10	5.87±0.29	6.68±0.13	6.27±0.37	6.57±0.03	6.24±0.49
	HS+HT S1	6.82±0.05	5.93±0.45	6.75±0.11	5.78±0.05	6.87±0.09	6.54±0.18	6.78±0.02	6.11±0.38
	HS+HT S2	6.90±0.04	5.98±0.47	6.87±0.13	5.80±0.07	7.00±0.10	6.67±0.24	6.78±0.18	6.14±0.48
	HS+HT S3	6.44±0.06	6.26±0.24	6.38±0.09	5.78±0.07	6.48±0.05	6.52±0.07	6.46±0.18	6.43±0.08
	HT+HS C	6.62±0.11	6.01±0.35	6.60±0.10	5.73±0.03	6.71±0.07	6.48±0.11	6.62±0.06	6.42±0.38
	HT+HS S1	6.79±0.13	5.93±0.40	6.76±0.10	5.83±0.05	6.88±0.06	6.60±0.15	6.79±0.03	6.30±0.43
	HT+HS S2	6.88±0.08	6.03±0.42	6.88±0.11	5.87±0.08	7.02±0.06	6.69±0.24	6.88±0.07	6.20±0.46
	HT+HS S3	6.62±0.21	6.02±0.26	6.39±0.09	5.97±0.19	6.46±0.03	6.46±0.14	6.43±0.04	6.45±0.10
Ca^{++}	HS+HT C	60.00±8.51	207.92±78.95	53.83±27.35	228.33±80.60	80.00±24.75	147.00±122.59	68.00±11.59	164.33±95.06
	HS+HT S1	27.50±4.15	150.33±66.45	26.67±15.02	206.6±5.16	39.17±15.54	59.33±33.14	31.75±6.69	118.17±54.70
	HS+HT S2	31.00±5.10	150.42±69.75	26.00±14.39	208.33±7.53	34.17±8.98	53.67±28.65	34.83±8.14	130.50±66.89
	HS+HT S3	28.33±3.87	37.42±21.92	26.17±14.58	89.33±3.50	43.83±17.10	23.83±11.65	34.58±6.76	31.92±10.78
	HT+HS C	53.83±13.90	174.92±93.18	54.00±25.61	296.67±18.62	81.00±30.42	97.00±32.61	69.33±11.18	138.67±73.64
	HT+HS S1	37.17±15.14	177.58±81.17	25.33±13.89	200.00±15.49	39.83±17.94	53.67±21.63	32.25±6.81	102.67±66.53
	HT+HS S2	30.08±5.16	138.58±65.26	26.83±14.22	190.00±20.98	35.83±11.63	52.50±26.51	36.42±7.88	130.25±70.14
	HT+HS S3	29.92±4.08	92.42±84.26	27.33±14.57	63.00±23.40	43.00±20.22	25.50±12.11	35.83±5.72	33.50±11.77
DS	HS+HT C	0.34±0.11	0.37±0.05	0.49±0.25	0.37±0.09	0.46±0.15	1.15±1.20	0.40±0.14	0.51±0.14
	HS+HT S1	0.31±0.12	0.34±0.09	0.52±0.37	0.30±0.05	0.44±0.14	1.23±1.24	0.42±0.28	0.63±0.49
	HS+HT S2	0.31±0.10	0.36±0.10	0.50±0.23	0.31±0.07	0.51±0.18	0.71±0.41	0.34±0.12	0.45±0.14
	HS+HT S3	0.33±0.08	0.35±0.10	0.50±0.33	0.31±0.09	0.50±0.20	1.885±1.31	0.442±0.15	0.56±0.28
	HT+HS C	0.61±0.15	0.3465±0.08	0.73±0.27	0.35±0.14	0.70±0.21	1.74±1.94	0.48±0.13	0.58±0.36
	HT+HS S1	0.54±0.11	0.28±0.06	0.65±0.19	0.37±0.11	0.40±0.10	0.93±0.70	0.75±0.42	0.63±0.39
	HT+HS S2	0.54±0.15	0.34±0.10	0.707±0.24	0.32±0.11	0.53±0.11	0.88±0.57	0.61±0.24	0.96±1.06
	HT+HS S3	0.49±0.09	0.37±0.12	0.65±0.24	0.376±0.19	0.76±0.34	2.08±1.88	0.50±0.15	0.68±0.14
D (4,3)	HS+HT C	0.17±0.04	0.19±0.05	0.19±0.04	0.19±0.05	0.43±0.24	2.52±1.96	0.22±0.03	0.24±0.05
	HS+HT S1	0.19±0.07	0.22±0.07	0.21±0.04	0.33±0.28	0.59±0.37	1.92±2.35	0.28±0.07	0.44±0.45
	HS+HT S2	0.20±0.06	0.25±0.06	0.23±0.02	0.23±0.03	1.13±0.90	1.58±1.46	0.25±0.06	0.53±0.57
	HS+HT S3	0.24±0.11	1.74±1.74	0.19±0.06	0.36±0.15	0.50±0.30	4.52±4.40	0.41±0.29	2.43±1.60
	HT+HS C	1.43±0.74	0.46±0.21	1.762±0.41	0.50±0.15	2.48±2.14	1.38±1.25	0.95±0.94	0.76±1.03
	HT+HS S1	1.51±0.77	0.31±0.07	1.25±0.37	0.30±0.04	6.58±12.96	7.72±0.02	0.78±1.07	0.76±0.98
	HT+HS S2	0.90±0.56	0.33±0.09	1.19±0.67	0.59±0.57	1.47±1.17	1.83±1.05	0.53±0.01	0.80±0.75

	HT+HS S3	1.83±1.38	1.50±1.61	1.61±0.47	1.19±1.14	2.77±1.79	4.55±3.61	1.70±1.63	3.81±2.448
D (3,2)	HS+HT C	0.11±0.00	0.11±0.00	0.116±0.00	0.11±0.00	0.13±0.01	0.13±0.01	0.12±0.00	0.11±0.00
	HS+HT S1	0.12±0.01	0.12±0.01	0.12±0.01	0.12±0.01	0.13±0.01	0.14±0.03	0.12±0.01	0.12±0.01
	HS+HT S2	0.12±0.01	0.12±0.01	0.12±0.01	0.12±0.01	0.14±0.02	0.15±0.03	0.12±0.01	0.12±0.00
	HS+HT S3	0.11±0.01	0.13±0.01	0.11±0.01	0.11±0.00	0.13±0.01	0.19±0.08	0.12±0.00	0.14±0.01
	HT+HS C	0.12±0.00	0.12±0.01	0.12±0.00	0.12±0.00	0.13±0.01	0.14±0.01	0.12±0.00	0.12±0.00
	HT+HS S1	0.12±0.00	0.12±0.00	0.12±0.00	0.12±0.00	0.13±0.01	0.14±0.02	0.12±0.01	0.12±0.02
	HT+HS S2	0.12±0.00	0.12±0.00	0.12±0.00	0.12±0.00	0.14±0.01	0.15±0.03	0.12±0.00	0.12±0.01
	HT+HS S3	0.13±0.01	0.13±0.01	0.12±0.00	0.12±0.02	0.15±0.02	0.21±0.08	0.12±0.01	0.15±0.02

Table 22: Seasonal analysis Results of processed skimmed milk collected over the period May 2017 to February 2018, and the effect of seasonality and treatments (addition DSHP (S1), TSC (S2), mixture SDHP and DSHP (S3), HS and HT) on pH, ionic Ca, sedimentation rate and particle size distribution on the first day and 30 days from processing.(Results are mean± Standard deviation).

5.1-pH

The pH of processed skimmed milk recorded a significant variation ($p<0.05$) when the analysis of variance was carried out against the seasons, treatment and the interaction between seasons and treatment are presented in Table 23. Table 23 shows the relationship between treatment and storage time, according to the Tukey method and 95% Confidence, the pH value for all treatment on D1 are different from D2 of the same samples. The highest average pH value recorded in day one (D1) in autumn for samples (HS+HT)-S2 and (HT+HS)-S2, 7.00±0.10 and 7.02±0.06 respectively. The same samples recorded the lowest pH.

Table 23: Analysis of variance seasons, treatment, days and their interaction on processed skimmed milk pH, Ca^{++}, particle size distribution D (3,2) and D (4,3) and dry sedimentation percentage (DS%) on D1 and d D2 from processing. DSHP= (S1), TSC= (S2), SDHP and DSHP 2:1= (S3), high shear homogenization=HS, heat treatment= HT, day one=D1, and day thirty=D2

Variables	Source	DF	Adj SS	Adj MS	F-Value	P-Value
pH	Seasons	3	9.34	3.11338	52.47	0.000
	Treatment	15	44.758	2.98387	50.29	0.000
	Seasons*Treatment	45	9.281	0.20624	3.48	0.000
	Error	512	30.378	0.05933		
	Total	575	99.398			
Ca^{++}	Seasons	3	152819	50940	26.21	0.000
	Treatment	15	1523615	101574	52.27	0.000
	Seasons*Treatment	45	378564	8413	4.33	0.000
	Error	512	994956	1943		
	Total	575	3224596			
D (3,2)	Seasons	3	0.04884	0.016282	80.56	0.000
	Treatment	15	0.03903	0.002602	12.87	0.000
	Seasons*Treatment	45	0.03246	0.000721	3.57	0.000
	Error	490	0.09904	0.000202		
	Total	553	0.21162			
D (4,3)	Seasons	3	237.3	79.1	20.11	0.000
	Treatment	15	338	22.53	5.73	0.000
	Seasons*Treatment	45	304.2	6.76	1.72	0.003
	Error	490	1927.1	3.93		
	Total	553	2781.6			
DS%	Seasons	3	19.445	6.4817	33.61	0.000
	Treatment	15	8.793	0.5862	3.04	0.000
	Seasons*Treatment	45	27.915	0.6203	3.22	0.000
	Error	504	97.19	0.1928		
	Total	567	151.25			

value in summer on D2, 5.80±0.10 and 5.87±0.10 respectively. The lowest pH was noted for the control (C) sample on D2 in summer 5.73±0.03 (Table 22). Autumn of D1 samples showed the higher

pH value than other seasons for HT+HS-S2, HS+HT-S2 while for D2, most of the milk samples in autumn recorded the higher pH value (Figures 26 and 27).

Table 24 presents the results when the pH value of the skimmed milk is analysed for the interaction between seasons and treatment. The results show that all treatment in spring and summer revealed differences between D1 and D2 samples. In winter, treatments with S1 and S2 recorded difference between D1 and D2, while no differences were recorded in autumn.

Table 24: Grouping Information using the Tukey method and 95% confidence and mean, for the relationship between treatment and days of storage of processed skimmed pH, Ca, D (4,3), D (3,2) and DS% on D1 and d D2 from processing. Samples with different letters are significantly different at $p<0.05$. DSHP= (S1), TSC= (S2), SDHP and DSHP 2:1= (S3), high shear homogenization=HS, heat treatment= HT, day one=D1, and day thirty=D2

Treatment	pH	Ca^{++}	(4,3)	D (3,2)	DS%	pH	Ca^{++}	D (4,3)	D (3,2)4	DS%
HS+HT C-D1	6.61	65.46	0.25	0.12	0.42	A	A	A	A	A
HS+HT C-D2	6.07	186.90	0.79	0.12	0.60	B	B	A	A	A
HT+HS C-D1	6.64	64.54	1.65	0.12	0.63	A	A	A	A	A
HT+HS C-D2	6.16	176.81	1.65	0.12	0.76	B	B	A	A	A
HS+HT S1-D1	6.80	31.27	0.32	0.12	0.42	A	A	A	A	A
HS+HT S1-D2	6.09	133.63	0.73	0.12	0.63	B	B	A	A	B
HT+HS S1-D1	6.80	33.65	2.53	0.12	0.59	A	A	A	A	A
HT+HS S1-D2	6.16	133.48	2.28	0.12	0.55	B	B	A	A	A
HS+HT S2-D1	6.89	31.50	0.45	0.12	0.41	A	A	A	A	A
HS+HT S2-D2	6.15	135.73	0.65	0.13	0.46	B	B	A	A	A
HT+HS S2-D1	6.91	32.29	1.02	0.12	0.60	A	A	A	A	A
HT+HS S2-D2	6.20	127.83	0.89	0.13	0.62	B	B	A	A	A
HS+HT S3-D1	6.44	33.23	0.33	0.12	0.44	A	A	A	A	A
HS+HT S3-D2	6.24	45.63	2.26	0.14	0.78	A	A	B	B	A
HT+HS S3-D1	6.47	34.02	1.98	0.13	0.60	A	A	A	A	A
HT+HS S3-D2	6.22	53.60	2.76	0.15	0.88	B	A	A	B	A

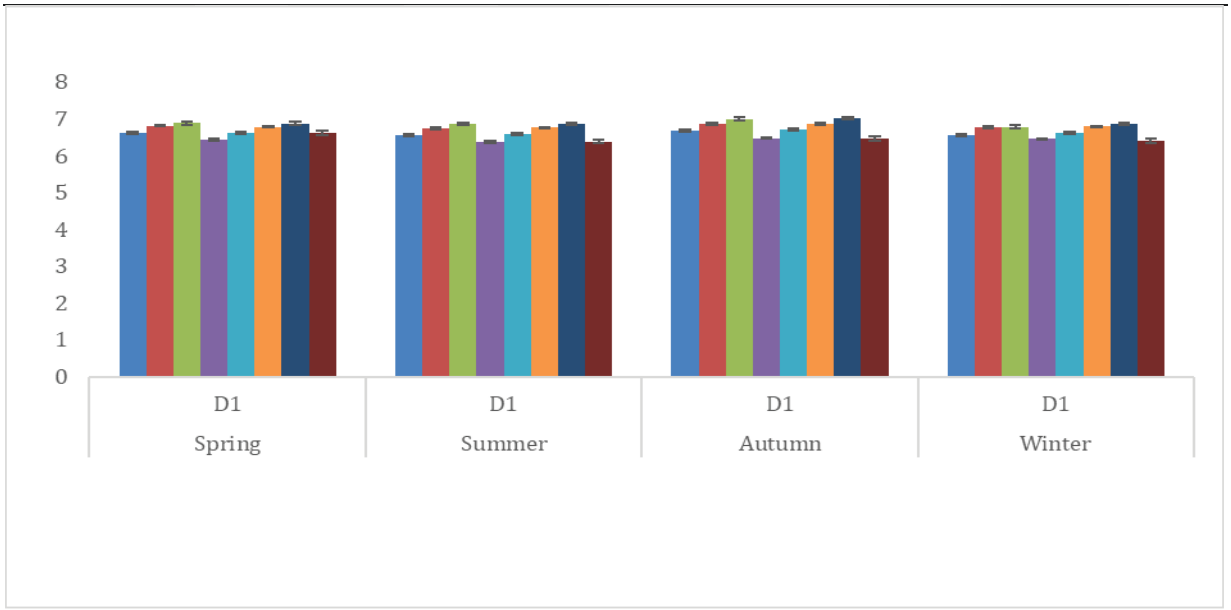

Figure 26: Seasonal pH value for processed skimmed milk on D1 from processing collected over the period March 2017 to February 2018 (Results are mean and standard deviation). DSHP= S1, TSC= S2, SDHP and DSHP 2:1= S3, high shear homogenization=HS, heat treatment= HT, day one=D1.

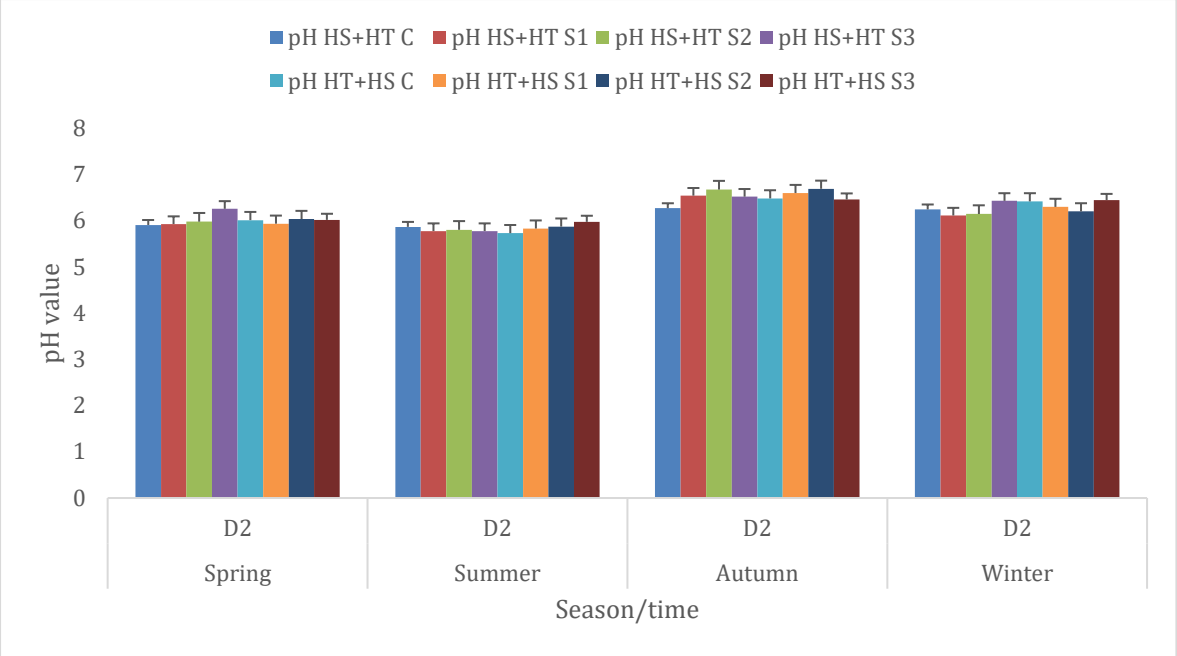

Figure 27: Seasonal pH value for processed skimmed milk on D2 from processing collected over the period March 2017 to February 2018 (Results are mean and standard deviation). DSHP= S1, TSC= S2, SDHP and DSHP 2:1= S3, high shear homogenization=HS, heat treatment= HT, day thirty=D2

5.2-Free calcium ion (Ca^{++})

When the analysis of variance for the mean concentration of Ca^{++} in processed skimmed was performed, the results showed a significant difference ($p<0.05$) (Table 23). The analysis of variance was done against seasons, treatment and the interaction of seasons and treatment, and results noted a significant variation for all three factors (Table 23).

Table 25: Analysis of the interaction of season and treatment using Tukey Pairwise comparisons, for the pH of processed skimmed milk on D1 and D2, (Grouping information using the Tukey method and 95% confidence). DSHP= S1, TSC= S2, SDHP and DSHP 2:1= S3, high shear homogenization=HS, heat treatment= HT, day one=D1, and day thirty=D2

Seasons	Seasons*Treatment	Mean	Significant
Spring	HS+HT C-D1	6.62	A
	HS+HT C-D2	5.91	B
	HT+HS C-D1	6.62	A
	HT+HS C-D2	6.01	B
	HS+HT S1-D1	6.82	A
	HS+HT S1-D2	5.93	B
	HT+HS S1-D1	6.79	A
	HT+HS S1-D2	5.93	B
	HS+HT S2-D1	6.9	A
	HS+HT S2-D2	5.98	B
	HT+HS S2-D1	6.88	A
	HT+HS S2-D2	5.98	B
	HT+HS S3-D1	6.62	A
	HT+HS S3-D2	6.02	B
Summer	HT+HS C-D1	6.6	A
	HT+HS C-D2	5.73	B
	HS+HT C-D1	6.55	A

	HS+HT C-D2	5.87	B
	HT+HS S1-D1	6.76	A
	HT+HS S1-D2	5.83	B
	HS+HT S1-D1	6.75	A
	HS+HT S1-D2	5.78	B
	HT+HS S2-D1	6.88	A
	HT+HS S2-D2	5.87	B
	HS+HT S2-D1	6.87	A
	HS+HT S2-D2	5.8	B
	HS+HT S3-D1	6.38	A
	HS+HT S3-D2	5.78	B
Winter	HT+HS S1-D2	6.3	A
	HT+HS S1-D1	6.79	B
	HS+HT S1-D2	6.11	A
	HS+HT S1-D1	6.78	B
	HT+HS S2-D1	6.88	A
	HT+HS S2-D2	6.2	B
	HS+HT S2-D1	6.78	A
	HS+HT S2-D2	6.14	B

The highest mean concentration of Ca^{++} was recorded in summer, 296.67±18.62mg/L for HT+HS-C samples on D2, while the lowest mean concentration was recorded in autumn for HS+HT-S3 for D2, and HT+HS-S1 on D1 for summer 23.83±11.65mg/L and 25.13.89±13.89mg/L respectively.

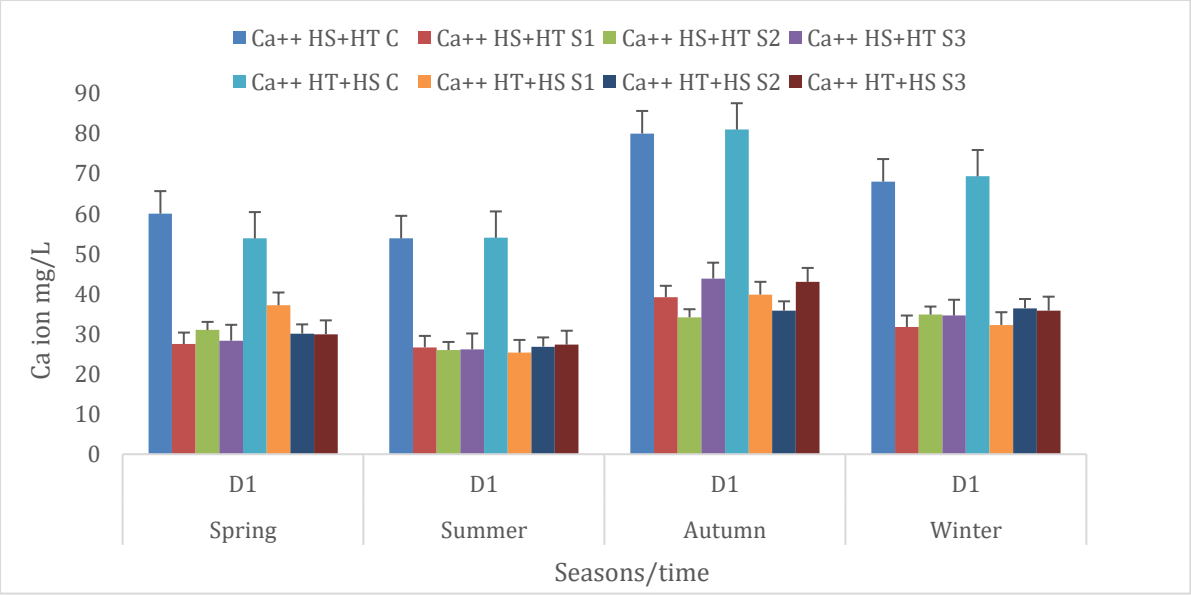

Figure 28: Seasonal difference of calcium ion (Ca^{++}) concentration for processed skimmed milk on D1 from processing collected over the period March 2017 to February 2018 (Results are mean and standard deviation). DSHP= S1, TSC= S2, SDHP and DSHP 2:1= S3, high shear homogenization=HS, heat treatment= HT, day one=D1.

The mean concertation of Ca++ for all control samples was highest for both D1 and D2.

When analysis was done against treatment, a difference was recorded between D1 and D2 for most samples. The results of the analysis revealed that the concentration of Ca++

increases with storage time. No difference was noted for samples processed using salt S3 (Table 24). The concentration of Ca++ for all samples collected in autumn showed higher levels on D1, and D2 for summer samples (Figures 28 and 29). The concentration of Ca++ for all samples increased with the increase of storage time. In autumn and winter, the Ca++ concentration declined only for samples that were processed using S3 (Table 21) but did not show any significant differences (Table 24). When analysis was done against the interaction between seasons and treatment, a difference was recorded in spring, summer, and winter (Table 26). All samples processed using C, S1 and S2 for the three seasons were found to be quite different from each other. D1 samples were found to be different from D2.

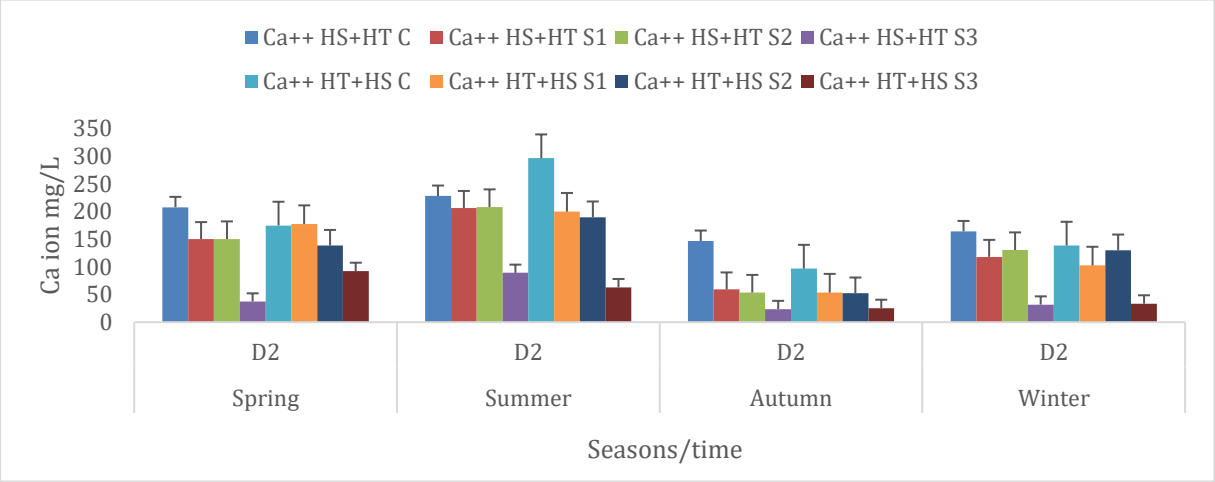

Figure 29: Seasonal variation of calcium ion (Ca^{++}) concentration for processed skimmed milk on D30 from processing collected over the period March 2017 to February 2018 (Results are mean and standard deviation). DSHP= S1, TSC= S2, SDHP and DSHP 2:1= S3, high shear homogenization=HS, heat treatment= HT, day thirty=D2

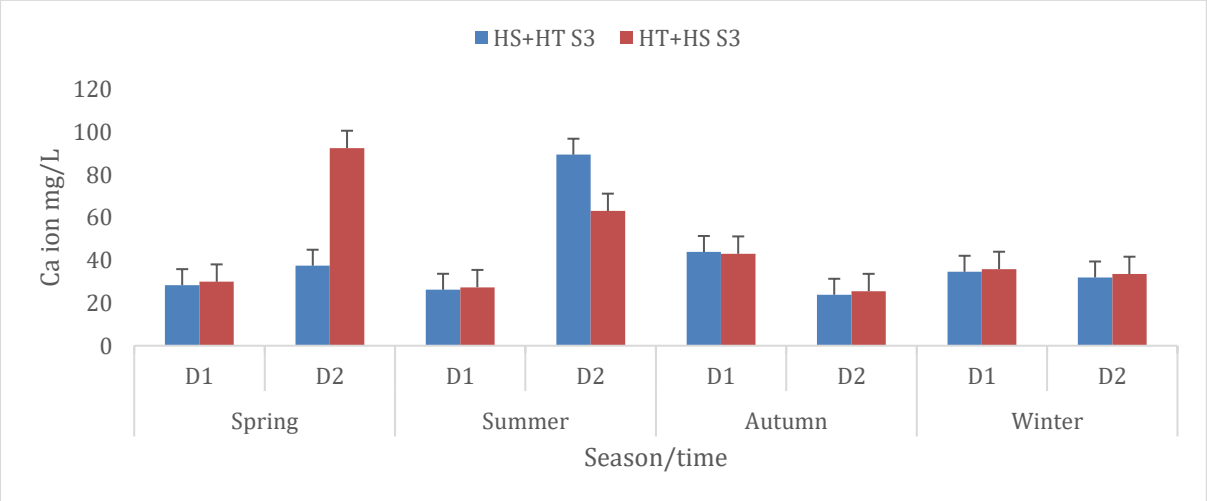

Figure 30: Seasonal variation calcium ion (Ca^{++}) concentration for HT+HS-S3 and HS+HT-S3 on D1 and D2 from processing. (Results are mean and standard deviation). DSHP= S1, TSC= S2, SDHP and DSHP 2:1= S3, high shear homogenization=HS, heat treatment= HT, day one=D1, and day thirty=D2

Table 26: Analysis of variance for the interaction season and treatment using Tukey Pairwise comparisons, for the Ca^{++} of processed skimmed milk on D1 and D2, (Grouping information using the Tukey method and 95% confidence) for milk collected over the period May 2017 to February 2018. Samples with different letters are significantly different at p<0.05. DSHP= S1, TSC= S2, SDHP and DSHP 2:1= S3, high shear homogenization=HS, heat treatment= HT, day one=D1, and day thirty=D2

Seasons	Seasons*Treatment	Mean	Significant
Spring	HS+HT C-D1	60	A
	HS+HT C-D2	207.92	B
	HT+HS C-D1	53.83	A
	HT+HS C-D2	174.92	B
	HS+HT S1-D1	27.5	A
	HS+HT S1-D2	150.33	B
	HT+HS S1-D1	37.17	A
	HT+HS S1-D2	177.58	B
	HS+HT S2-D1	31	A
	HS+HT S2-D2	150.42	B
	HT+HS S2-D1	30.08	A
	HT+HS S2-D2	138.58	B
Summer	HS+HT C-D1	53.83	A
	HS+HT C-D2	228.33	B
	HT+HS C-D1	54	A
	HT+HS C-D2	296.67	B
	HS+HT S1-D1	26.67	A
	HS+HT S1-D2	206.67	B
	HT+HS S1-D1	25.33	A
	HT+HS S1-D2	200	B
	HS+HT S2-D1	26	A
	HS+HT S2-D2	208.33	B
	HT+HS S2-D1	26.83	A
	HT+HS S2-D2	190	B
Winter	HS+HT C-D1	68	A
	HS+HT C-D2	164.33	B
	HS+HT S1-D1	31.75	A
	HS+HT S1-D2	118.17	B
	HS+HT S2-D1	34.83	A
	HS+HT S2-D2	130.5	B
	HT+HS S2-D1	36.42	A
	HT+HS S2-D2	130.25	B

5.3- Particle size distribution (PSD)

The particle size distribution D (3,2) and D (4,3) of processed skimmed milk recorded was significantly different (p<0.005), between seasons, treatment and there were interactions (table 23). The highest average mean D (4,3) values were recorded in autumn (2.77µm) for sample HT+HS-S3 on D1. The same sample recorded the highest average mean D (4,3) in autumn (4.55µm) on D2 (Table 22). The lowest average particle size D (4,3) values were recorded in winter for D1 and in summer for D2 (1.70µm) for sample HT+HS-S3 (Table 22).

When analysed against treatment for both D (4,3) and D (3,2), HS+HT-S1-D1 was found to be different to HT+HS-S1-D1 and HT+HS-S1-D2, but similar to HS+HT-S1-D2, while the sample HS+HT-S3-D1 was found to be different in all samples treated using the same salt for D (4,3). There was no difference between HS+HT-S3-D2, HT+HS-S3-D1, and HT+HS-S3-D1. The mean particle size distribution for HS+HT-S3-D1 was found to be smaller when compared to other samples with the same salt. HS+HTS3-D1 and HT+HS-S3-D2 was found to be different to HS+HTS3-D2 and HT+HS-S3-D1 for D (3,2).

When analysed for interactions between seasons and treatment, a difference was recorded in autumn only (Table 26). For D (3,2), HS+HT-C-D2 was different to HT+HS-C-D1 and HS+HT-C-D1 but similar to HT+HS-C-D2, while for S3 samples HS+HT and HT+HS in D1 was found to be different to HS+HT and HT+HS in D2. D (4,3), HS+HT-S1-D2 was found to be different to HT+HS-S1-D1 and HS+HT-S1-D1 but similar to HT+HS-S1-D2.

5.4-Dry sedimentation (DS%)

The rate of sedimentation (DS%) recorded a significant variation ($p<0.005$) when analysed against seasons, treatment and their interaction. Table 22 shows a substantial difference between each factor and their interactions.

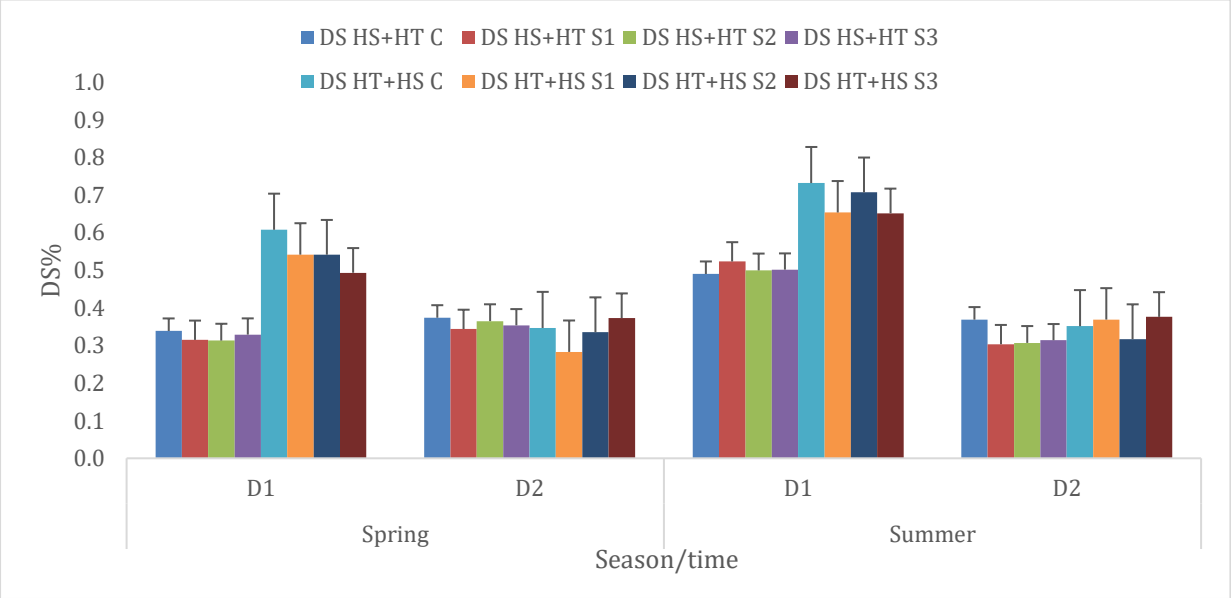

Figure 31: Seasonal variation in dry sedimentation percentage (DS%) for processed skimmed milk on D1 and D2 from processing collected over the period March 2017 to February 2018 (Results are mean and standard deviation). DSHP= S1, TSC= S2, SDHP and DSHP 2:1= S3, high shear homogenization=HS, heat treatment= HT, day one=D1, and day thirty=D2

The Figures 28 and 29 show the rate of DS% in spring and summer on D2 were lower than D1, while for autumn and winter were higher in D2 than D1. Autumn was found to be different to all the three seasons with the highest rate of sedimentation of 0.93%, while spring and summer were similar to each other, with spring recording the lowest sedimentation rate of 0.39%. The results also showed

no difference between winter and summer. Table 24 shows the analysis of DS% to the treatment.

The only difference recorded was between HT+HS-S3-D2 and HS+HT-S3-D1. Autumn samples in D2 were highest for DS% compared to other seasons.

Table 27: Analysis of variance for the interaction season and treatment using Tukey Pairwise comparisons, for the DS and particles size distribution of processed skimmed milk on D1 and D2, (Grouping information using the Tukey method and 95% confidence) for milk collected over the period May 2017 to February 2018. Samples with different letters are significantly different at $p<0.05$. DSHP= S1, TSC= S2, SDHP and DSHP 2:1= S3, high shear homogenization=HS, heat treatment= HT, day one=D1, and day thirty=D2

Season	Variables	Seasons*Treatment	Mean	Significant
Autumn	DS%	HS+HT C-D1	0.46	A
		HS+HT C-D2	1.15	B
		HT+HS C-D1	0.7	A
		HT+HS C-D2	1.74	B
		HS+HT S3-D1	0.5	A
		HS+HT S3-D2	1.89	B
		HT+HS S3-D1	0.76	A
		HT+HS S3-D2	2.08	B
	D (3,2)	HT+HS C-D1	0.7	A
		HT+HS C-D2	1.74	B
		HS+HT S3-D2	1.89	A
		HS+HT S3-D1	0.5	B
		HT+HS S3-D1	0.76	A
		HT+HS S3-D2	2.08	A

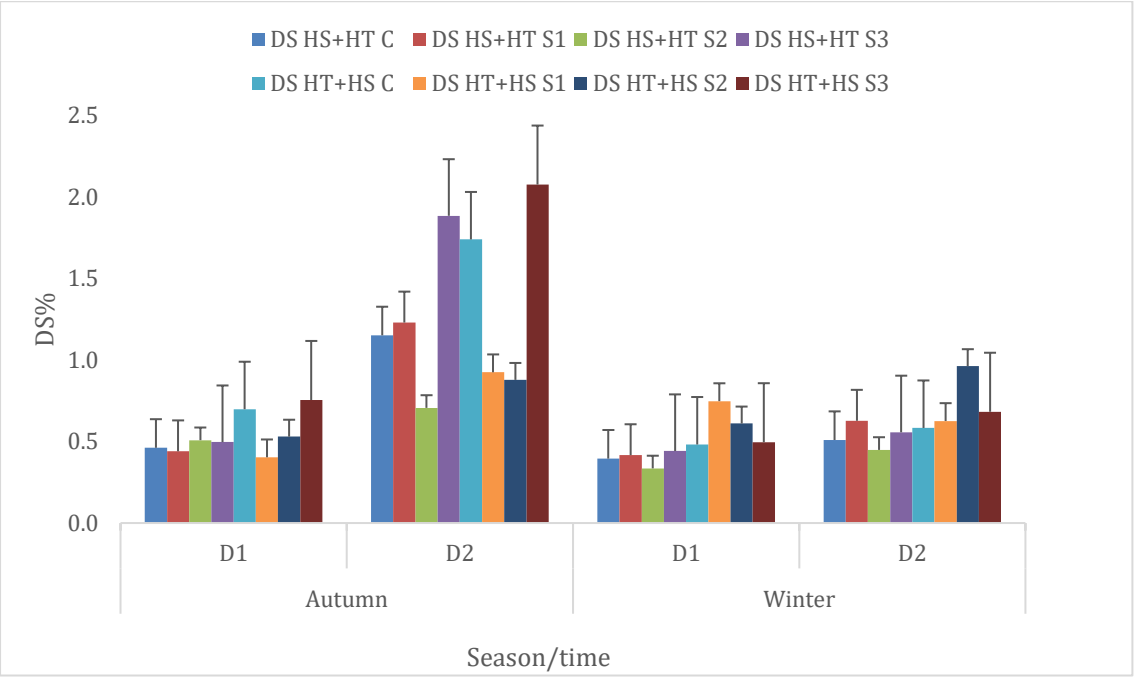

Figure 32: Seasonal variation in dry sedimentation percentage (DS%) for processed skimmed milk on D1 and D2 from processing collected over the period March 2017 to February 2018 (Results are mean and standard deviation). DSHP= S1, TSC= S2, SDHP and DSHP 2:1= S3, high shear homogenization=HS, heat treatment= HT, day one=D1, and day thirty=D2.

When interactions between seasons and treatment were analysed, a difference was recorded in autumn only (Table 29). The rate of DS for HS+HT-C-D2 was found to be different to HT+HS-C-D1 and HS+HT-C-D1 but similar to HT+HS-C-D2. For S3 samples, HS+HT and HT+HS in D1 was found to be different to HS+HT and HT+HS in D2.

5.5- Colour

Based on Hunters' system, which is a 3-dimensional rectangular (L*, a*, and b*), the colour of processed skim milk was measured. The letters characterise the colours, where L* (lightness), it ranges between 0-100, where 0 is black, and 100 is white, a* signify red – green.

Table 28: analysis of variance for Hunter L*, a*, b*, Huge angle, chroma and ΔE values of processed skim milk. Result of analysis of variance for seasons, treatment and the interaction between season and treatment on D1 and D2. DSHP= S1, TSC= S2, SDHP and DSHP 2:1= S3, high shear homogenization=HS, heat treatment= HT, day one=D1, and day thirty=D2

Variables	Source	DF	Adj SS	Adj MS	F-Value	P-Value
L*	Seasons	3	12048	4016.14	71.56	0.000
	Treatment	15	7848	523.18	9.32	0.000
	Seasons*Treatment	45	3583	79.63	1.42	0.040
	Error	512	28734	56.12		
	Total	575	53467			
a*	Seasons	3	1266.5	422.16	68.28	0.000
	Treatment	15	182.1	12.14	1.96	0.020
	Seasons*Treatment	45	796.8	17.71	2.86	0.000
	Error	512	3165.6	6.18		
	Total	575	5426.6			
b*	Seasons	3	7805	2601.61	156.05	0.000
	Treatment	15	2166	144.39	8.66	0.000
	Seasons*Treatment	45	1937	43.05	2.58	0.000
	Error	512	8536	16.67		
	Total	575	20822			
Huge	Seasons	3	31860	10619.9	20.57	0.000
	Treatment	15	43454	2897	5.61	0.000
	Seasons*Treatment	45	31956	710.1	1.38	0.058
	Error	512	264313	516.2		
	Total	575	390812			
Chroma	Seasons	3	6250.5	2083.5	123.14	0.000
	Treatment	15	997.2	66.48	3.93	0.000
	Seasons*Treatment	45	2872.9	63.84	3.77	0.000
	Error	512	8662.6	16.92		
	Total	575	18684.8			
ΔE	Seasons	3	9341	3113.66	48.11	0.000
	Treatment	7	747.8	106.83	1.65	0.122
	Seasons*Treatment	21	510	24.28	0.38	0.995
	Error	256	16568.6	64.72		
	Total	287	27492.3			

A positive value denotes red; negative value green, and zero neutral, while the letter b* represents blue-yellow. The positive benefits are yellow, negative values blue, and zero neutral. The angle Hue

is the quality of a visual sensation. According to this an area appears to be similar to one of the alleged colours, often refers red, green, blue, and yellow. Chroma is the colourfulness of an area referred to as a ratio of the brightness of a similarly illuminated area that appears white. The relationship between colourfulness and chroma is similar to the relationship between brightness and lightness. Samples were assessed for the seasons, treatment and the interaction between the seasons and the treatment, on D1 and D2 for L*, a*, b*, Huge angle, chroma and total colour (ΔE). The analysis of variance results carried out to changes in colour for the seasons, treatment, and the interaction between the seasons and the treatment, on D1 and D2 f, for L*, a*, b*, Huge angle and chroma, recorded a significant variation ($p<0.05$) as in Table 28.

ΔE was found to be significant to season only but not different to treatment and the interaction between seasons and treatment. The interaction between season and treatment for Huge also showed no variation($p>0.05$) (Table 28). When L*, a*, b*, Huge angle, chroma and ΔE are analysed for seasonal variation, using Tukey Pairwise comparisons, L* and b* were found to be no different in spring and summer, while during winter and autumn were found to be different to each other. a* noted a difference in winter but not to other seasons (Figure 33).

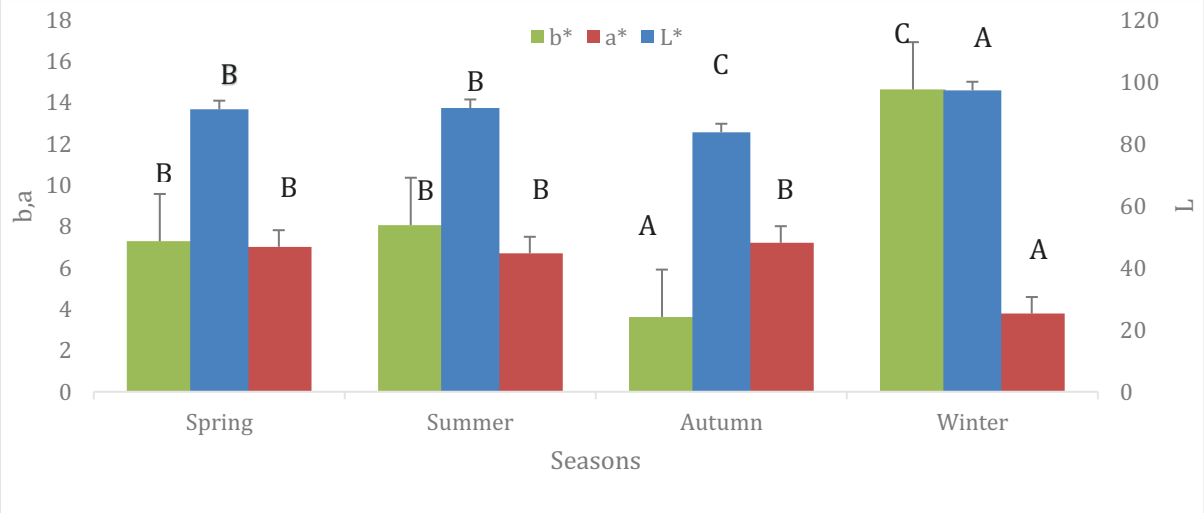

Figure 33: Seasonal variation analysis of colour change for Hunter L*, a*, and b* in processed skimmed milk on D1 and D2, (Results are grouping information using the Tukey method and 95% confidence, and standard deviation) for milk collected over the period May 2017 to February 2018. Samples with different letters are significantly different at $p<0.05$. DSHP= S1, TSC= S2, SDHP and DSHP 2:1= S3, high shear homogenization=HS, heat treatment= HT, day one=D1, and day thirty=D2. L*= lightness, a*= red – green and b*= blue-yellow.

Huge angle was found to be different in autumn but not to the other seasons, while chroma was not different between spring and summer, while winter and autumn were different to each other and so was between summer and spring (Figure 34). Spring and autumn were found to be different to each

other and to summer and winter, while summer and winter showed no difference to each other for ΔE (Figure 34).

Table 29 presents the results of colour change in L*, a*, b*, Huge angle, chroma when samples were analysed for the interaction of treatment and seasons using Tukey Pairwise comparisons. The L* values for samples HT+HS-S1-D1 was found to be different from HS+HT-S1-D2 in autumn, while HS+HT-S3-D1 and HT+HS-S3-D1 were to be similar but different from HT+HS-S3-D2 in winter. The rest of the samples were not different.

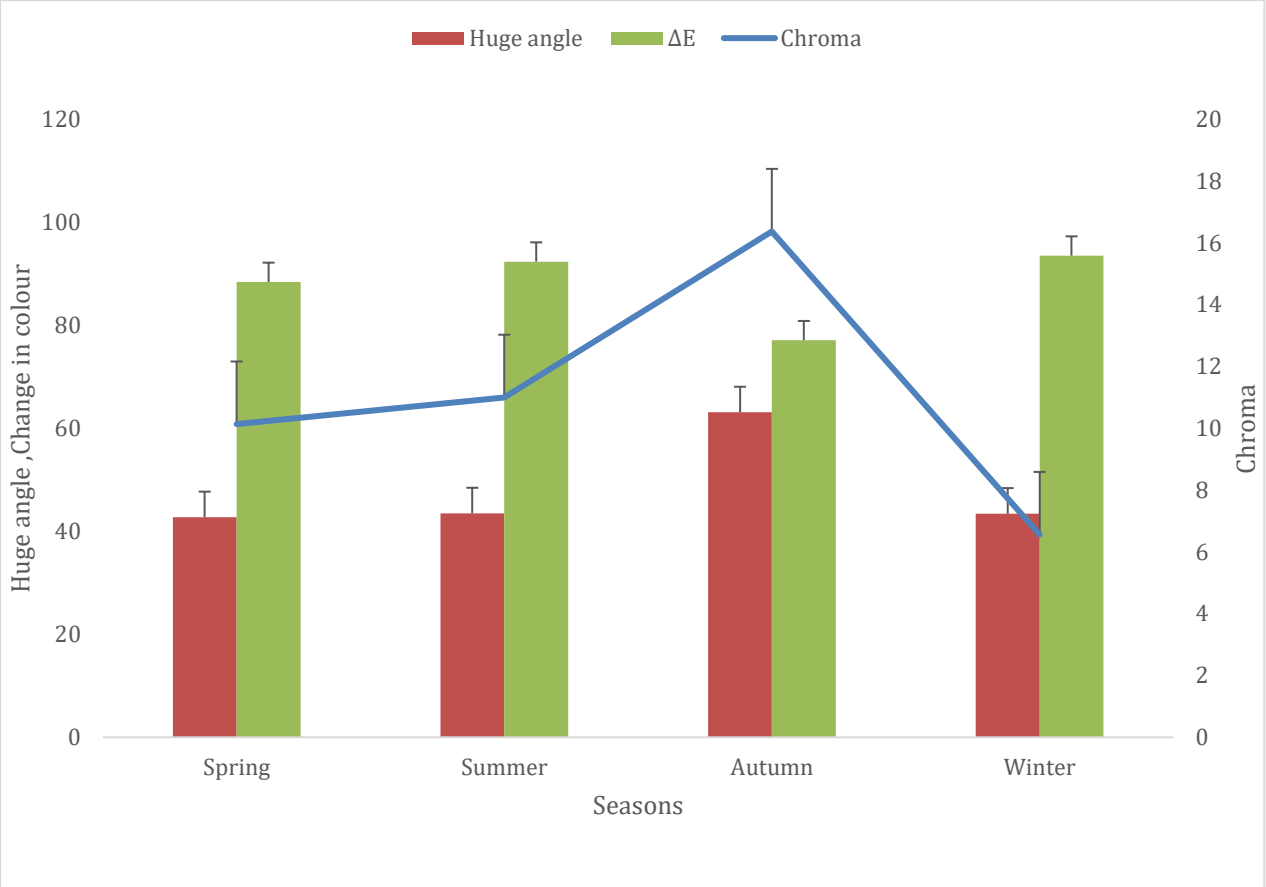

Figure 34: Seasonal variation analysis of colour change for Huge angle, chroma and ΔE in processed skimmed milk on D1 and D2, (Results are grouping information using the Tukey method and 95% confidence, and standard deviation) for milk collected over the period May 2017 to February 2018. Chroma is presented in the secondary axis. Samples with different letters are significantly different at $p<0.05$. Huge angle value is negative. DSHP= S1, TSC= S2, SDHP and DSHP 2:1= S3, high shear homogenization=HS, heat treatment= HT, day one=D1, and day thirty=D2.

The colour change in a* for the same factors also recorded, HS+HT-S3-D1 and HT+HS-S3-D1 are different to HT+HS-S3-D2. The same analysis for colour change in b* shows, HT+HS-S2-D1 and HS+HT-S2-D1 in summer were different to the same samples on D2 and in spring for sample HT+HS-S3-D1. Chroma recorded differences in spring between HS+HT-S1-D2 and HT+HS-S1-D1 and in summer between HS+HT-S2-D2 and HT+HS-S2-D1. Huge angle and ΔE were not different to the interaction between treatment and seasons.

Table 29: Analysis of variance for the interaction between season and treatment using Tukey Pairwise comparisons, for colour change in Hunter L*, a*, b*, Huge angle and chroma for processed skimmed milk on D1 and D2, (Grouping information using the Tukey method and 95% confidence) for milk collected over the period May 2017 to February 2018. Samples with different letters are significantly different at p<0.05, when compared to days of storage. DSHP= S1, TSC= S2, SDHP and DSHP 2:1= S3, high shear homogenization=HS, heat treatment= HT, day one=D1, and day thirty=D2

Variables	Treatment	Mean	Significant
L*	HS+HT C-D1	96.39	A
	HS+HT C-D2	89.85	B
	HT+HS C-D1	96.36	A
	HT+HS C-D2	88.95	B
	HS+HT S2-D1	93.12	A
	HS+HT S2-D2	86.69	B
	HT+HS S2-D1	92.03	A
	HT+HS S2-D2	86.96	B
	HS+HT S3-D1	95.04	A
	HS+HT S3-D2	84.97	B
	HT+HS S3-D1	93.93	A
	HT+HS S3-D2	84.57	B
b*	HS+HT S2-D1	10.92	A
	HS+HT S2-D2	5.93	B
	HT+HS S2-D1	11.22	A
	HT+HS S2-D2	5.48	B
	HS+HT S3-D1	10.26	A
	HS+HT S3-D2	5.83	B
	HT+HS S3-D1	10.81	A
	HT+HS S3-D2	6.3	B
Chroma	HS+HT S2-D1	12.92	A
	HS+HT S2-D2	9.28	B
	HT+HS S2-D1	13.34	A
	HT+HS S2-D2	9.34	B
Huge angle	HT+HS S3-D1	-53.03	A
	HT+HS S3-D2	-31.08	B
	HS+HT S3-D1	-54	A
	HS+HT S3-D2	-27.72	B

Table 29 presents the result of colour change in L*, a*, b*, Huge angle, chroma and ΔE when samples were analysed for treatment using Tukey Pairwise comparisons. The results present the colour change in L*, samples HT+HS -C-D1 and HS+HT-C-D1 were different to the same samples in D2, while HS+HT-S1-D1 were different to the same sample in D2.

Table 30: Analysis of variance for the treatment for colour change in Hunter L*, a*, b*, Huge angle and chroma for processed skimmed milk on D1 and D2, (Grouping information using the Tukey method and 95% confidence) for milk collected over the period May 2017 to February 2018. Samples with different letters are significantly different at $p<0.05$. DSHP= S1, TSC= S2, SDHP and DSHP 2:1= S3, high shear homogenization=HS, heat treatment= HT, day one=D1, and day thirty=D2

Variables	Treatments	Mean	Significant
L*	HS+HT C-D1	96.39	A
	HS+HT C-D2	89.85	B
	HT+HS C-D1	96.36	A
	HT+HS C-D2	88.95	B
	HS+HT S2-D1	93.12	A
	HS+HT S2-D2	86.69	B
	HS+HT S3-D1	95.04	A
	HS+HT S3-D2	84.97	B
	HT+HS S3-D1	93.93	A
	HT+HS S3-D2	84.57	B
b*	HS+HT S2-D1	10.92	A
	HS+HT S2-D2	5.93	B
	HT+HS S2-D1	11.22	A
	HT+HS S2-D2	5.48	B
	HS+HT S3-D1	10.26	A
	HS+HT S3-D2	5.83	B
	HT+HS S3-D2	6.3	A
	HT+HS S3-D1	10.81	B
Chroma	HS+HT S2-D1	12.92	A
	HS+HT S2-D2	9.28	B
	HT+HS S2-D2	9.34	A
	HT+HS S2-D1	13.34	B
Huge angle	HS+HT S3-D1	-54	A
	HS+HT S3-D2	-27.72	B
	HT+HS S3-D1	-53.03	A
	HT+HS S3-D2	-31.08	B

A similar difference was also recorded for samples with S3, where HS+HT-S3-D1 and HT+HS-S3-D1 were different to HT+HS-S3-D2 and HS+HT-S3-D2. The rest of the samples rather than the one in table 27 did not show any difference to L*. The table also presents difference in b*, samples with salts S2 and S3 on D1 were found to be different from D2, while sample HT+HS-S1-D2 was different to HS+HT-S1-D1. When chroma analysis was conducted, samples HS+HT-S2-D1 and HT+HS-S2-D1 were found to be different from same samples in D2. Huge angle noted differences in samples processed using S2 and S3 on D1 to the same samples in D2.

Table 31: The relationship between seasons, treatment, days and their interaction of colour change in Hunter L*, a*, b*, Huge angle, chroma and ΔE on processed skimmed milk colour change on D1 and D2, milk collected from May 2017 to February 2018. DSHP= S1, TSC= S2, SDHP and DSHP 2:1= S3, high shear homogenization=HS, heat treatment= HT, day one=D1, and day thirty=D2

Season	Treatment	L*-D1	L*-D2	a*-D1	a*-D2	b*-D1	b*-D2	Hue angle-D1	Hue angle-D2	Chroma-D1	Chroma-D2	ΔE
Spring	HS+HT C	96.69	90.77	-6.79	-5.39	9.77	5.73	-55.26	-46.05	11.91	7.89	90.97
	HS+HT S1	95.13	90.22	-7.58	-5.22	10.41	3.49	-52.22	-32.51	12.90	6.32	90.60
	HS+HT S2	94.22	87.11	-7.66	-6.08	10.89	4.25	-54.18	-33.53	13.33	7.47	87.52
	HS+HT S3	95.24	84.34	-7.87	-6.39	10.75	2.25	-52.15	-16.27	13.34	7.15	84.88
	HT+HS C	94.78	91.03	-6.88	-5.03	9.79	5.12	-53.13	-43.69	11.98	7.23	91.30
	HT+HS S1	93.31	88.46	-7.94	-5.82	10.61	4.86	-50.03	-38.23	13.30	7.64	88.88
	HT+HS S2	92.50	86.57	-8.20	-5.56	10.91	3.89	-50.85	-33.08	13.70	6.95	87.02
	HT+HS S3	92.66	85.27	-8.19	-6.54	10.91	2.90	-51.10	-21.11	13.69	7.33	85.91
Summer	HS+HT C	93.05	92.72	-7.62	-4.32	11.62	3.02	-56.73	-34.04	13.89	5.31	93.18
	HS+HT S1	92.01	94.85	-8.30	-5.74	12.14	4.65	-55.62	-37.59	14.70	7.45	95.19
	HS+HT S2	89.32	91.83	-8.52	-4.99	13.01	2.48	-56.79	-24.35	15.55	5.68	92.51
	HS+HT S3	91.83	90.99	-8.61	-6.38	12.41	3.61	-55.24	-28.19	15.10	7.40	91.46
	HT+HS C	93.59	91.71	-7.90	-5.09	12.09	5.36	-56.82	-44.60	14.44	7.46	92.02
	HT+HS S1	90.98	94.44	-8.56	-5.26	12.62	4.43	-55.86	-39.02	15.24	6.92	94.86
	HT+HS S2	89.22	90.14	-8.94	-6.13	13.34	2.69	-56.18	-18.27	16.05	7.49	90.88
	HT+HS S3	89.97	88.01	-8.73	-7.16	12.75	2.89	-55.59	-20.49	15.46	7.83	88.59
Autumn	HS+HT C	93.26	80.31	-6.69	-6.89	12.93	15.69	-62.64	-65.64	14.62	17.14	80.87
	HS+HT S1	90.36	72.80	-7.15	-7.66	13.75	15.84	-62.39	-61.40	15.61	17.70	73.55
	HS+HT S2	88.51	74.73	-8.04	-7.12	14.44	14.92	-60.98	-63.70	16.60	16.54	75.31
	HS+HT S3	91.39	75.14	-7.37	-7.32	13.32	15.92	-61.26	-65.58	15.30	17.52	75.78
	HT+HS C	93.77	76.99	-6.58	-7.01	13.65	14.42	-64.32	-59.07	15.17	16.20	77.79
	HT+HS S1	92.18	76.66	-6.60	-7.44	12.93	15.65	-62.56	-63.27	14.58	17.38	77.75
	HT+HSS2	86.54	77.63	-8.28	-6.50	15.40	13.57	-61.82	-64.14	17.52	15.04	78.07
	HT+HS S3	91.97	76.95	-7.16	-7.53	14.87	16.78	-64.32	-66.10	16.51	18.40	77.42
Winter	HS+HT C	102.56	95.61	-1.49	-4.19	3.57	3.61	-63.30	-49.80	4.24	7.46	95.90
	HS+HT S1	101.19	95.65	-2.70	-5.17	4.90	3.91	-40.66	-47.39	5.69	7.54	96.01
	HS+HT S2	100.43	93.10	-3.10	-5.15	5.35	2.08	-64.51	-39.97	6.22	7.44	93.40
	HS+HT S3	101.70	89.40	-2.31	-6.78	4.55	1.53	-47.36	-0.84	5.25	8.31	89.98
	HT+HS C	103.29	96.07	-1.45	-3.67	3.74	3.40	-39.36	-51.24	4.52	7.94	96.47

	HT+HS S1	100.85	93.44	-2.37	-4.44	4.81	2.16	-43.94	-42.59	5.47	6.67	93.77
	HT+HSS2	99.88	93.49	-2.99	-5.14	5.22	1.78	-64.98	-40.73	6.08	7.89	93.85
	HT+HS S3	101.13	88.05	-2.60	-6.95	4.70	2.63	-41.09	-16.61	5.47	8.78	88.60

5.6- Principle Component Analysis (PCA)

Principle component analysis (PCA) was carried out on the data of processed skimmed milk collected during May 2017 and February 2018. The data set consisted of 23 samples and 24 variables. The PCA similarity map defined by principal components PC 1 and PC 2 showed a discrimination of samples between different seasons. On PCA, the similarity map is defined by PC1 and PC2. Milk samples were separated according to PC1 with a variation of 37.79% (Figure 29). Autumn processed skimmed milk show discrimination from milk of the other seasons with a variation of 37.79% (Figure 29). Milk from summer, spring and winter showed only a small variation. The PCA in Figure 29 clearly shows the effect of the milk compositions following processing. All samples from autumn and majority of spring and summer were located on the positive part of the similarity map, whereas samples from winter and few from spring winter are on the negative part (Figure 35).

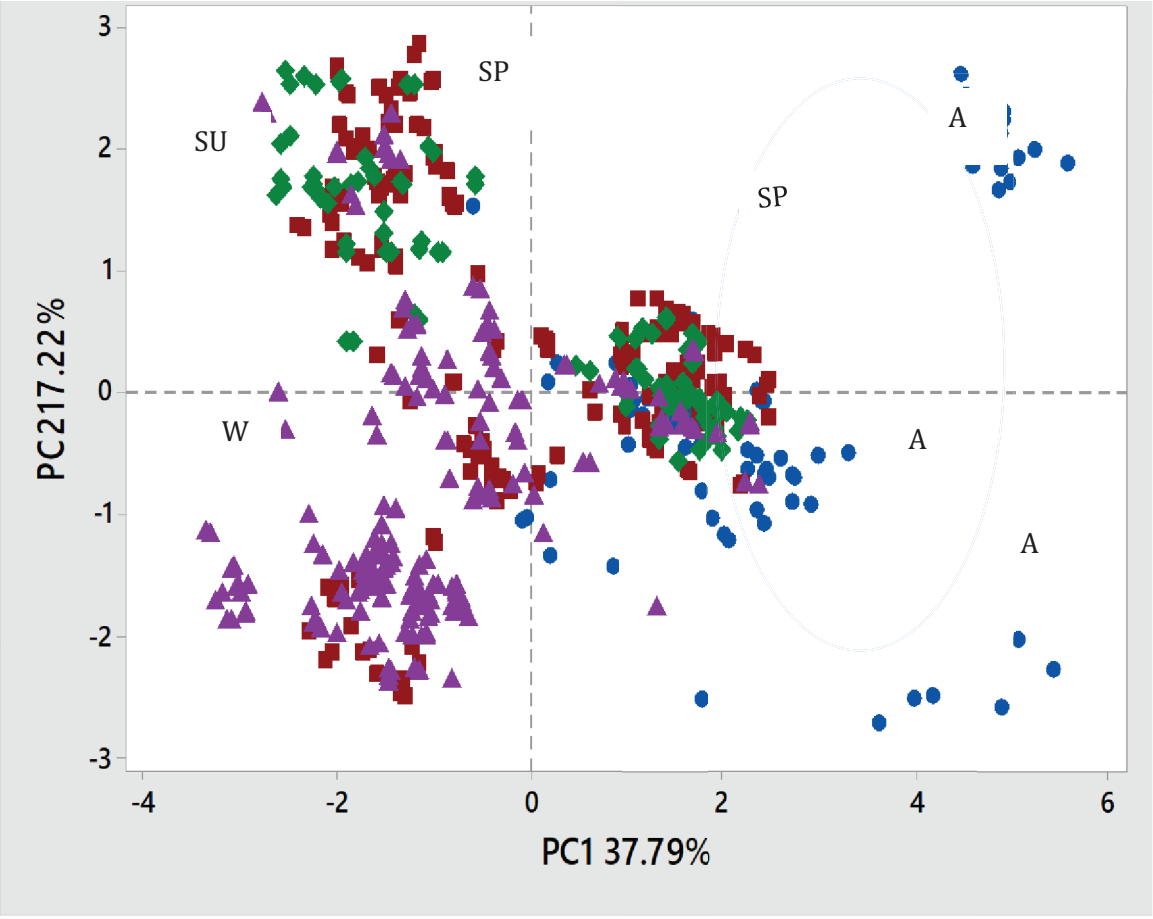

Figure 35: Seasonal variation on processed skimmed milk according principle component analysis (PCA). A= autumn, SP= spring, SU= summer and W= winter

5.6-Discussion

Stabilising salts are added to milk to improve heat stability (Sweetsur & Muir., 1980). According to Sweetsur and Muir. (1980), DSHP (S1 in the study) and TSC (S2 in the study) should be used if the natural pH of milk is acidic. Zadow (1978) stated that pH of cow milk is > 6.62, with a small amount of

sediment formed during heat treatment, which contradicted my findings. This study exposed a drop in the pH value in summer milk to 6.55 for HS+HT-C and winter milk to 6.57 for HS+HT-C. Chen et al. (2012) reported that addition of stabilizing salts to milk increased pH and decreased Ca^{++}, resulted in low sedimentation which agrees with the Ca^{++} concentration values but contradicts with the pH and dry sedimentation results of my study. Heat treatment of milk results in the transfer of the soluble Ca^{++} and soluble P to the colloidal phase, with a decrease in milk pH (Pouliot, Boulet, & Paquin., 1989) which agrees with the finding of my study. Van Boekel et al. (1989) stated that milk pH decreases with increasing temperature and time.

Based on the analysis of variance, a seasonal variation was detected ($p<0.05$) for processed skim milk pH on D1 and D2 following processing. Samples from spring, summer and autumn are different ($p<0.05$) to each other but not different ($p>0.05$) between winter and summer on D1, while D2 sample from all seasons found to be different ($p<0.05$) to each other. A marked decline in pH value was recorded in all D2 samples in summer. The pH value on D1 increased for samples treated with S1 and S2 which agrees with the findings of (Chen et al., 2012; Zadow, 1978), but decreased in the control sample, while for sample with S3 no variation ($p>0.05$) between the sample of different treatment. The highest pH value was recorded on D1 in autumn for samples HS+HT-S2 and HT+HS-S2 with values of 7.00±0.10 and 7.02±0.06 respectively. The pH of the same samples dropped to 6.67±0.24 and 6.69±0.24 on D2 from processing. All samples contained S1 and S2 recorded a higher pH than the C and S3 samples on D1(Chen et al., 2012; Zadow, 1978). The lowest pH was noted for the HS+HT-C and HT+HS-C on D2 in summer 5.73±0.03 (Table 21). The pH value of all samples declined on D2.

Seasonal variation ($p < 0.05$) was noted in the concentration of Ca (Van Boekel et al., 1989). The spring and summer samples were found to be similar ($p>0.05$) to each other but different ($p<0.05$) to autumn and winter for on D1, while D2 values between seasons were different ($p < 0.05$) to each other. The level of Ca^{++} concentration on D1 for most of the samples declined after heat treatment for all seasons which is in agreement with the findings of (Chen et al., 2012; Zadow, 1978). The highest mean concentration of Ca^{++} was recorded in summer 296.67±18.62mg/L for HT+HS-C on D2 at a pH of 5.73±0.03, while the lowest mean concentration was recorded in autumn for HS+HT-S3 on D2, 23.83±11.65mg/L at a pH of 6.52±0.07. The more acidic the milk is the higher concentration of Ca^{++}, while the more neutral the milk, the less concentration of Ca^{++} (Chen et al., 2012). As noted by Van Boekel et al. (1989), the Ca^{++} concentration of milk depends on milk pH before heating. The increase in soluble Ca or Ca^{++} activity did not occur during heating. However, at constant temperature (20°C), a pH decrease was accompanied by an increase in Ca^{++}. The Ca^{++} concentration was restored following cooling of milk. In this study, the control sample recorded higher Ca^{++} on D2 which agrees with the findings of (Van Boekel et al., 1989). The mean concertation of Ca^{++} for all

control samples was highest on D2, while for the samples heated and the stabilizer added (S1, S2, and S3) the Ca^{++} concertation was seen to be less than the control sample which proves the addition of the salts had a significant impact in reducing the concentration of Ca^{++} after heat treatment which agrees with the findings of (Chen et al., 2012) . The Ca^{++} concentration of all samples increases with the decrease in pH on D2, except for the HS+HT-S3 sample of which the autumn concentration of both $Ca^{++}$and pH decreased. The strength of Ca^{++} for control sample was found to be different to samples processed with S1, S2 and S3. Samples prepared with S1 and S2, their Ca^{++} on D2 was found to be similar but different from the control sample and the S3 sample (figure 24), which showed both S1 and S2 has the same effect on reducing Ca^{++} which agrees with the findings of (Chen et al., 2012; Udabage, McKinnon, & Augustin, 2000).

As shown in Table 22, it appears that applying high shear would generate conditions during processing of milk that would govern protein interactions and their behaviour. Heating milk at and >85 ºC, most of the whey proteins would undergo reversible denaturation initiated by unfolding. Impact of shear stress at this temperature appeared more marked at high shear rates 11,000 rpm. The particle size distribution (D 3, 2) and D (4, 3) of processed skim milk recorded a significant variation ($p<0.005$) during seasons, treatment and their interaction for D1 and D2 (Table 23). The particle size distribution of skim milk was found to reduce when it is heated. Skim milk pH declines after heat treatment, similar to the particle size distribution. As noted in tables 8 and 22, the particle size distribution D (3, 2) and D (4,3) for the control sample declined with the declining pH of skim milk when heated. Anema and Li. (2003) had previously found that the particle size in skim milk increased when cooked which agrees with the finding of my study. The increase in particle size of skim milk is reported to be due to whey protein and especially β-lg (Anema & Li., 2003). On D2 the particle size, especially D (4, 3), increased its size and this phenomenon has been reported by (Christiansen, 2017). Autumn for D (3, 2)-FSM, D (3, 2)-D1 and D (3,2)-D2 were found to be significantly different ($p<0.005$) between all three seasons. For D (4, 3), samples in spring and summer were also found to be different ($p<0.005$) from autumn and winter but similar to each other for FSM, while for D1 in spring was found to be significantly different ($p<0.005$) to summer, autumn and winter. For D (4, 3)-D2 in spring was found to be significantly different ($p<0.005$) to summer and winter but not to autumn (Figure 26 and 27).

The highest average means D (4, 3) were recorded in autumn (2.77µm) for sample HT+HS-S3-D1. The same sample recorded the highest average mean D (4,3) in autumn (4.55µm) on D2 (Table 22). Autumn milk recorded the highest protein concentration (table 8). As reported by (Anema & Li., 2003) increase in milk particle size is due to whey protein. The lowest average particle size D (4, 3) were recorded in winter for D1 while in summer for D2 (1.70µm) for sample HT+HS-S3 (Table 22). As shown in Table 22, the D (4, 3) for samples high sheared before heat treatment increased on D2,

while for samples high sheared after heat treatment declined on D2. This confirms the effect of high shear on the milk particle size particularly on D (4, 3).

Analysis of variance against treatment for both D (4,3) and D (3,2), HS+HT-S1-D1 was found to be different to HT+HS-S1-D1 and HT+HS-S1-D2, but similar to HS+HT-S1-D2, while sample HS+HT-S3-D1 was found to be different to all sample treated using the same salt for D (4,3). The mean particle size distribution for HS+HT-S3-D1 was smaller when compared to other samples with the corresponding salt. HS+HTS3-D1 and HT+HS-S3-D2 were found to be different from HS+HTS3-D2 and HT+HS-S3-D1 for D (3, 2). On analysis of the interaction between seasons and treatment, a difference was recorded in autumn only (table 24). For D (3,2), HS+HT-S3-D1 was found to differ from HT+HS-S3-D1, while for D (4,3), HS+HT-S1-D1 was different from HT+HS-S1-D1. On analysis of interaction between seasons and treatment, a different was recorded in autumn only (Table 24). For D (3,2), HS+HT-S3-D1 was found to be different from HT+HS-S3-D1, while for D (4,3), HS+HT-S1-D1 was found to be different from HT+HS-S1-D1.

Heat-treatment of milk often generates aggregates which sediment or clump together on the surface depending on their size, specific weight, and electric charge. The aggregates are a result of the denatured protein, fat, lactose and inorganics (Datta, Elliott, Perkins, & Deeth., 2002). The rate of sedimentation depends on several factors including raw milk quality, type and severity of heat treatment, homogenization process, homogenizing pressure and storage temperature (Datta, Elliott, Perkins, & Deeth., 2002). The rate of dry sedimentation (DS %) recorded a significant variation ($p<0.005$) when analysed against seasons (Table 21). Figure 30 shows the rate of DS% FSM. Spring and summer samples showed no difference ($p>0.005$) to each other and similarly for spring to winter. For the same sample, summer, autumn and winter were found to be different from each other ($p<0.005$). Figure 30 also shows DS% for D1. Samples from spring and summer were different from each other, while summer samples were similar ($p>0.005$) to those from autumn. Values in spring was found to be the similar ($p>0.005$) to in winter, while autumn samples were similar ($p>0.005$) to the ones in winter for D1. D2 samples also showed similarity ($p>0.005$) between spring and summer, while both seasons were found to different ($p<0.005$) to autumn and winter.

The highest mean sedimentation was reordered in autumn 0.39% at pH of 6.66, and the lowest of 0.21% at pH of 6.71 in winter for FSM. The rate DS% in summer and spring on D2 were lower (0.35% and 0.34%) at pH 6.00 and 5.83 respectively, while D1 of the same seasons reported a mean sedimentation of 0.43% and 0.59% at pH 6.70 and 6.65 respectively. The highest sedimentation was in autumn in D2, 1.32% at pH value of 6.53. Samples in autumn were found to be different ($p<0.005$) during all three seasons with the highest mean sedimentation of 1.32%, while spring and summer were similar ($p>0.005$) to each other. Samples in summer recorded the lowest deposition of 0.34% for D2. When compared to FSM DS%, for processed samples, the rate of DS increased. FMS and D2

processed skim milk recorded the highest DS% in autumn, while D1 processed skim milk recorded its highest rate in summer.

Based on an analysis of variance, for DS% against the treatment, significant variation ($p<0.005$) between a sample subjected to the same salts but different treatment for D1 (Figure 31). All samples heated before high shearing recorded a higher DS% to samples heat treated after high shearing subjected to the same salt regime. The highest mean DS% was recorded for HT+HS-C on D1 0.63%, while the lowest mean was recorded to HS+HT-S2. On both D1and D2 0.41% and 0.46% at pH level of 6.89 and 6.15 respectively is in agreement with the findings of (Gaur, Schalk, & Anema., 2017). They reported that tri-sodium citrate (S2) was effective as it reduced Ca^{++} concentration and increased pH. On analysis, HS+HT-S2 for both D1 and D2 showed the lowest DS%. The addition of stabilizers (S1, S2 and S3) can maximize heat stability of milk (Sweetsur & Muir., 1980), and the addition of stabilizers decreased the rate of DS% in all samples when compared to the processed control samples.

The total colour change (ΔE) has previously been used as a measure for browning in milk by other authors (Chugh et al., 2014; Pagliarini, Vernile, & Peri., 1990). Based on the result of ANOVA, a change in colour for seasons, treatment, and the interaction between seasons and treatment, on D1 and D2, for L*, a*, b*, Huge angle, chroma and ΔE were observed with a significant variation of heat treatment, on seasons, treatment, and the interaction between seasons and treatment ($p<0.05$) on L*, a*, b*, and chroma (table 24), while a marked significant difference ($p<0.05$) was also noted for season and treatment but not ($p>0.05$) for the interaction between seasons and treatment. There was a significant effect of heat treatment on season ($p<0.05$) on ΔE but while no difference ($p>0.05$) was recorded for treatment and the interaction between treatment and seasons. There is a tendency of a slight decrease in ΔE for heat treated sample until 85◦C. An increase in brightening at lower heat treatments and shift toward darkening and yellow/brown colour at higher temperatures has previously been reported by Kessler and Fink. (1986).

When L*, a*, b*, Huge angle, chroma and ΔE were analysed for seasonal variation, using Tukey Pairwise comparisons, L* and b* were found to be not significant ($p>0.05$) between spring and summer, while winter values were different to autumn ($p<0.05$) and also summer different to spring. For a* a difference was observed only in winter (Figure 27).

Huge angle was found to be different ($p<0.05$) in autumn samples but not at other seasons, while chroma was found to have no difference between spring and summer, while winter values were different to autumn ($p<0.05$) and summer different spring (Figure 28).

The total colour change (ΔE), in spring was different to autumn and summer different to winter, while summer and winter values showed no difference to each other (Figure 28). Table 25 presents the results of a colour change in L*, a*, b*, Huge angle, chroma when samples were analysed for the interaction of treatment and seasons using Tukey Pairwise comparisons. The L* for samples HT+HS-

S1-D1 was found to be significantly different ($p<0.05$) to HS+HT-S1-D2 in autumn, while HS+HT-S3-D1 and HT+HS-S3-D1 were similar to each other but different from HT+HS-S3-D2 in winter. The rest of the samples were not different from each other.

The colour change in a* for the same factors also recorded, HS+HT-S3-D1 and HT+HS-S3-D1 to be significantly different ($p<0.05$) from HT+HS-S3-D2. The same analysis for colour change in b* showed that HT+HS-S2-D1 and HS+HT-S2-D1 in summer was the same in different samples on D2, while in spring for sample HT+HS-S3-D1. Chroma recorded differences in spring between HS+HT-S1-D2 and HT+HS-S1-D1 and in summer between HS+HT-S2-D2 and HT+HS-S2-D1. Huge angle and ΔE recorded no difference from the interaction between treatment and seasons. Table 28 presents the result of a colour change in L*, a*, b*, Huge angle, chroma and ΔE when samples were analysed for treatment using Tukey Pairwise comparisons. The results present the colour change in L*, samples HT+HS -C-D1 and HS+HT-C-D1 found to be different to the same samples in D2, while HS+HT-S1-D1 were found to be different to the same sample in D2. A similar difference was also recorded for samples with S3, where HS+HT-S3-D1 and HT+HS-S3-D1 were different from HT+HS-S3-D2 and HS+HT-S3-D2. The rest of the samples except for one in table 26 did not show any differences to L*. Table 26 also shows the difference in b*. Samples with salts S2 and S3 on D1 were found to be significantly different from D2, while sample HT+HS-S1-D2 was found to be different from HS+HT-S1-D1. When chroma analysis was carried, samples HS+HT-S2-D1 and HT+HS-S2-D1 were found to be different from the same samples in D2. Huge angle noted the difference to samples processed using S2 and S3 on D1 to the same samples in D2.

The significant correlation between processed skimmed milk components is shown in table 27. A a strong negative relationship was recorded between the Ca^{++} and pH, when the pH increased the concentration of Ca^{++} decreased. Ca^{++} recorded a strong positive relation to D (3, 2)/D (4, 3). Table 27 also reveals that there is significant but weak relationship between D (3,2)/ Ca^{++}, D (3,2)/DS%, D (4,3)/ Ca^{++}, D (4,3)/DS%, D (4,3)/pH and D (3,2)/pH.

Chapter 6

General Discussion Concusion and Future study

6.1- General Discussion

This study clearly showed a seasonal variation in milk protein and fat concentrations (Walker et al., 2007), pH and BC (Chen et al., 2014), TPL, total whey protein and a-Casein, Ca^{++}, FAs, minerals, the particle sizes D (3, 2) and D (4, 3), and also ES%, and DS%. No seasonal variation was evident for TS%. The cows produced the maximum milk in spring, while the fat and protein concentrations were lowest at that time. The protein and fat concentrations were higher at the beginning and the end of the lactation period when compared with the middle period (Bansal et al., 2009). No significant seasonal variation was evident for TS% which is in contrast to the findings of Lindmark-Månsson et al. (2003). A significant seasonal difference ($p<0.05$) was noted in milk Ca^{++} concentration

Seasonal effect on FAs composition was observed during the study which support the findings of Glover et al., (2012). In general, samples taken in summer were found to be different to other seasons for C16:00 and C18:00, while for C18:1 and USFA winter samples were different to those taken at other seasons. No difference was noted in CLA between winter and spring and between summer and autumn. Winter and spring samples were different to those from summer and autumn to CLA which is in agreement with Samková & Węglarz, (2012) findings. The highest total UNSFA were in winter at 117.4mg/g and it declined to its lowest in spring 90.74mg/g which agrees with the findings of Thomson & Poel, (2000).

This study showed that the average mineral (Ca, K, Mg, Na, P, Zn, and S) concentrations differ significantly during the seasons ($p<0.05$). Autumn recoded the highest concentration to most the minerals. The finding of Ca and Mg from my study is in contrast to the findings Bates & Prentice, (1996), Debry, (2001) and Mapekula, Chimonyo, Mapiye, & Dzama, (2011) who found that the average Ca and Mg were at their lowest concentration in autumn and the maximum in spring.

Stabilizing salts are added to milk to improve heat stability (Sweetsur & Muir., 1980). According to Sweetsur and Muir. (1980), DSHP (S1 in the study) and TSC (S2 in the study) should be used if the natural pH of milk is acidic. During the study it was noted that the addition of stabilizing salts to milk increased pH and decreased Ca^{2+} and there was less sedimentation.

Heat treatment of milk results in the transfer of the soluble Ca^{++} and soluble P to the colloidal phase which results in a decrease in milk pH (Pouliot et al., 1989) and also decrease of the particle size distribution. Van Boekel et al. (1989b) stated that milk pH also decreased with increasing temperature and time. The pH value on D1 from heat treatment increased for samples treated with

S1 and S2 which is in agreement of the findings of (Chen et al., 2012; Zadow, 1978). The pH in the control samples and S3 samples decreased between the samples subjected to different treatments including corresponding salts.

The Ca^{++} concentration on D1 declined in most of the samples after heat treatment during all seasons which is in agreement with the findings (Chen et al., 2012; Zadow, 1978). The more acidic the milk is, higher the concentration of Ca^{++} while the more neutral the milk is lower the concentration of Ca^{++} (Chen et al., 2012). Ionic Ca activity of milk depends on milk pH before heating (Van Boekel et al., 1989).

As the pH of skim milk declined, the particle size distribution also declined, after heat treatment. The rate of sedimentation depends on several factors including raw milk quality, type and severity of heat treatment, homogenization process, homogenizing pressure and storage temperature (Datta, Elliott, Perkins, & Deeth., 2002). The highest mean sedimentation was reordered in autumn, while the lowest was in winter for FSM. The rate DS% in summer and spring on D2 were lower. In autumn the highest sedimentation was observed on D2. The addition of stabilizers (S1, S2 and S3) decreased the rate of DS% in all samples when compared to a processed control sample (Sweetsur & Muir., 1980).

The total colour change (ΔE) in spring was different to those in autumn and summer.

The significant correlation was found to be between the Ca^{++} and pH, Ca^{++} and D (3, 2), D (4, 3), D (3,2)/Ca^{++}, D (3,2)/DS%, D (4,3)/Ca^{++}, D (4,3)/DS%, D (4,3)/pH and D (3,2)/pH.

6.2-Conclusion

This study revealed seasonal variation in milk compotation and physiochemical properties. No seasonal variation was noted in TS%. The maximum milk production in spring was linked to the lowest fat and protein content. The protein and fat levels tend to be higher at the beginning and at the end of the lactation period when compared with the mid-lactation period. No significant seasonal variation was evident for TS%.

Heat treatment of milk results in the transfer of the soluble Ca^{++}, and soluble P to the colloidal phase, resulting in a decrease in milk pH and particle size distribution. Milk pH decreased with increasing temperature and time.

The addition of stabilizer also had a significant effect the rate of sedimentation of skim milk. It increased pH and decreased Ca^{++}, and the sedimentation rate was less. A change in colour (ΔE) was also recorded as the storage time increased.

Significant correlation were noted between the $Ca^{++}$and pH, Ca^{++} and D (3, 2), D (4, 3), D (3,2)/Ca^{++}, D (3,2)/DS%, D (4,3)/Ca^{++}, D (4,3)/DS%, D (4,3)/pH and D (3,2)/pH.

6.3- Future study

Future research in this area could be too closely investigate the feeding regime used during different seasons. The age and breed type of the herd also need to be considered in future studies. The extreme weather changes between seasons is also another to focus on. Sometimes during the same season, extreme temperature changes can occur, especially now with climate change. Furthermore, the effect of different heat treatment of milk, to ascertain a full picture of the effect of each heating system on the milk composition requires close attention.

Chapter 7

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Appendix A

Summary of statistical analysis

Table A 1: - General Linear Model using Minitab 17: pH versus Seasons and treatment.

Method: -

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Seasons	Fixed	4	Autumn, Spring, Summer, Winter
Treatment	Fixed	16	HS+HT C-D1, HS+HT C-D2, HS+HT S1-D1, HS+HT S1-D2, HS+HT S2-D1, HS+HT S2-D2, HS+HT S3-D1, HS+HT S3-D2, HT+HS C-D1, HT+HS C-D2, HT+HS S1-D1, HT+HS S1-D2, HT+HS S2-D1, HT+HS S2-D2, HT+HS S3-D1, HT+HS S3-D2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Seasons	3	9.340	3.11338	52.47	0.000
Treatment	15	44.758	2.98387	50.29	0.000
Seasons*Treatment	45	9.281	0.20624	3.48	0.000
Error	512	30.378	0.05933		
Total	575	99.398			

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	6.4283	0.0108	597.16	0.000	
Seasons					
Autumn	0.2167	0.0206	10.51	0.000	1.95

Spring	-0.0694	0.0164	-4.22	0.000	1.75
Summer	-0.1919	0.0206	-9.31	0.000	1.95
Treatment					
HS+HT C-D1	0.1785	0.0417	4.28	0.000	2.11
HS+HT C-D2	-0.3581	0.0417	-8.59	0.000	2.11
HS+HT S1-D1	0.3746	0.0417	8.98	0.000	2.11
HS+HT S1-D2	-0.3402	0.0417	-8.16	0.000	2.11
HS+HT S2-D1	0.4594	0.0417	11.02	0.000	2.11
HS+HT S2-D2	-0.2798	0.0417	-6.71	0.000	2.11
HS+HT S3-D1	0.0106	0.0417	0.25	0.799	2.11
HS+HT S3-D2	-0.1842	0.0417	-4.42	0.000	2.11
HT+HS C-D1	0.2073	0.0417	4.97	0.000	2.11
HT+HS C-D2	-0.2696	0.0417	-6.47	0.000	2.11
HT+HS S1-D1	0.3764	0.0417	9.03	0.000	2.11
HT+HS S1-D2	-0.2646	0.0417	-6.35	0.000	2.11
HT+HS S2-D1	0.4846	0.0417	11.62	0.000	2.11
HT+HS S2-D2	-0.2304	0.0417	-5.53	0.000	2.11
HT+HS S3-D1	0.0410	0.0417	0.98	0.326	2.11
Seasons*Treatment					
Autumn	-0.1435	0.0798	-1.80	0.073	3.87
HS+HT C-D1					
Autumn	-0.0185	0.0798	-0.23	0.817	3.87
HS+HT C-D2					
Autumn	-0.1512	0.0798	-1.89	0.059	3.87
HS+HT S1-D1					
Autumn	0.2352	0.0798	2.95	0.003	3.87
HS+HT S1-D2					
Autumn	-0.1010	0.0798	-1.27	0.206	3.87
HS+HT S2-D1					

Autumn HS+HT S2-D2	0.3065	0.0798	3.84	0.000	3.87
Autumn HS+HT S3-D1	-0.1739	0.0798	-2.18	0.030	3.87
Autumn HS+HT S3-D2	0.0575	0.0798	0.72	0.472	3.87
Autumn HT+HS C-D1	-0.1406	0.0798	-1.76	0.079	3.87
Autumn HT+HS C-D2	0.1046	0.0798	1.31	0.191	3.87
Autumn HT+HS S1-D1	-0.1448	0.0798	-1.81	0.070	3.87
Autumn HT+HS S1-D2	0.2163	0.0798	2.71	0.007	3.87
Autumn HT+HS S2-D1	-0.1146	0.0798	-1.44	0.152	3.87
Autumn HT+HS S2-D2	0.2738	0.0798	3.43	0.001	3.87
Autumn HT+HS S3-D1	-0.2227	0.0798	-2.79	0.005	3.87
Spring HS+HT C-D1	0.0859	0.0637	1.35	0.178	3.28
Spring HS+HT C-D2	-0.0949	0.0637	-1.49	0.137	3.28
Spring HS+HT S1-D1	0.0857	0.0637	1.35	0.179	3.28
Spring HS+HT S1-D2	-0.0920	0.0637	-1.44	0.149	3.28
Spring HS+HT S2-D1	0.0784	0.0637	1.23	0.219	3.28

Spring HS+HT S2-D2	-0.1016	0.0637	-1.60	0.111	3.28
Spring HS+HT S3-D1	0.0696	0.0637	1.09	0.275	3.28
Spring HS+HT S3-D2	0.0811	0.0637	1.27	0.203	3.28
Spring HT+HS C-D1	0.0496	0.0637	0.78	0.436	3.28
Spring HT+HS C-D2	-0.0785	0.0637	-1.23	0.218	3.28
Spring HT+HS S1-D1	0.0521	0.0637	0.82	0.413	3.28
Spring HT+HS S1-D2	-0.1610	0.0637	-2.53	0.012	3.28
Spring HT+HS S2-D1	0.0382	0.0637	0.60	0.549	3.28
Spring HT+HS S2-D2	-0.0943	0.0637	-1.48	0.139	3.28
Spring HT+HS S3-D1	0.2192	0.0637	3.44	0.001	3.28
Summer HS+HT C-D1	0.1350	0.0798	1.69	0.091	3.87
Summer HS+HT C-D2	-0.0133	0.0798	-0.17	0.868	3.87
Summer HS+HT S1-D1	0.1340	0.0798	1.68	0.094	3.87
Summer HS+HT S1-D2	-0.1212	0.0798	-1.52	0.129	3.87
Summer HS+HT S2-D1	0.1725	0.0798	2.16	0.031	3.87

Summer HS+HT S2-D2	-0.1550	0.0798	-1.94	0.053	3.87
Summer HS+HT S3-D1	0.1279	0.0798	1.60	0.110	3.87
Summer HS+HT S3-D2	-0.2773	0.0798	-3.47	0.001	3.87
Summer HT+HS C-D1	0.1529	0.0798	1.92	0.056	3.87
Summer HT+HS C-D2	-0.2385	0.0798	-2.99	0.003	3.87
Summer HT+HS S1-D1	0.1504	0.0798	1.88	0.060	3.87
Summer HT+HS S1-D2	-0.1435	0.0798	-1.80	0.073	3.87
Summer HT+HS S2-D1	0.1556	0.0798	1.95	0.052	3.87
Summer HT+HS S2-D2	-0.1360	0.0798	-1.70	0.089	3.87
Summer HT+HS S3-D1	0.1142	0.0798	1.43	0.153	3.87

Table A 2: General Linear Model using Minitab 17: Ca^{++} versus Seasons and treatment

Method: -

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Seasons	Fixed	4	Autumn, Spring, Summer, Winter

Treatment	Fixed	16	HS+HT C-D1, HS+HT C-D2, HS+HT S1-D1, HS+HT S1-D2, HS+HT S2-D1, HS+HT S2-D2, HS+HT S3-D1, HS+HT S3-D2, HT+HS C-D1, HT+HS C-D2, HT+HS S1-D1, HT+HS S1-D2, HT+HS S2-D1, HT+HS S2-D2, HT+HS S3-D1, HT+HS S3-D2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Seasons	3	152819	50940	26.21	0.000
Treatment	15	1523615	101574	52.27	0.000
Seasons*Treatment	45	378564	8413	4.33	0.000
Error	512	994956	1943		
Total	575	3224596			

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	82.47	1.95	42.33	0.000	
Seasons					
Autumn	-25.64	3.73	-6.87	0.000	1.95
Spring	6.74	2.98	2.27	0.024	1.75
Summer	26.81	3.73	7.19	0.000	1.95
Treatment					
HS+HT C-D1	-17.01	7.55	-2.25	0.025	2.11
HS+HT C-D2	104.42	7.55	13.84	0.000	2.11
HS+HT S1-D1	-51.20	7.55	-6.79	0.000	2.11
HS+HT S1-D2	51.15	7.55	6.78	0.000	2.11
HS+HT S2-D1	-50.97	7.55	-6.76	0.000	2.11
HS+HT S2-D2	53.26	7.55	7.06	0.000	2.11
HS+HT S3-D1	-49.24	7.55	-6.53	0.000	2.11
HS+HT S3-D2	-36.85	7.55	-4.88	0.000	2.11

HT+HS C-D1	-17.93	7.55	-2.38	0.018	2.11
HT+HS C-D2	94.34	7.55	12.50	0.000	2.11
HT+HS S1-D1	-48.83	7.55	-6.47	0.000	2.11
HT+HS S1-D2	51.01	7.55	6.76	0.000	2.11
HT+HS S2-D1	-50.18	7.55	-6.65	0.000	2.11
HT+HS S2-D2	45.36	7.55	6.01	0.000	2.11
HT+HS S3-D1	-48.45	7.55	-6.42	0.000	2.11
Seasons*Treatment					
Autumn	40.2	14.4	2.78	0.006	3.87
HS+HT C-D1					
Autumn	-14.3	14.4	-0.99	0.324	3.87
HS+HT C-D2					
Autumn	33.5	14.4	2.32	0.021	3.87
HS+HT S1-D1					
Autumn	-48.7	14.4	-3.37	0.001	3.87
HS+HT S1-D2					
Autumn	28.3	14.4	1.96	0.051	3.87
HS+HT S2-D1					
Autumn	-56.4	14.4	-3.91	0.000	3.87
HS+HT S2-D2					
Autumn	36.2	14.4	2.51	0.012	3.87
HS+HT S3-D1					
Autumn	3.8	14.4	0.27	0.790	3.87
HS+HT S3-D2					
Autumn	42.1	14.4	2.91	0.004	3.87
HT+HS C-D1					
Autumn	-54.2	14.4	-3.75	0.000	3.87
HT+HS C-D2					
Autumn	31.8	14.4	2.20	0.028	3.87
HT+HS S1-D1					

Autumn HT+HS S1-D2	-54.2	14.4	-3.75	0.000	3.87
Autumn HT+HS S2-D1	29.2	14.4	2.02	0.044	3.87
Autumn HT+HS S2-D2	-49.7	14.4	-3.44	0.001	3.87
Autumn HT+HS S3-D1	34.6	14.4	2.40	0.017	3.87
Spring HS+HT C-D1	-12.2	11.5	-1.06	0.290	3.28
Spring HS+HT C-D2	14.3	11.5	1.24	0.216	3.28
Spring HS+HT S1-D1	-10.5	11.5	-0.91	0.362	3.28
Spring HS+HT S1-D2	10.0	11.5	0.86	0.388	3.28
Spring HS+HT S2-D1	-7.2	11.5	-0.63	0.530	3.28
Spring HS+HT S2-D2	7.9	11.5	0.69	0.491	3.28
Spring HS+HT S3-D1	-11.6	11.5	-1.01	0.313	3.28
Spring HS+HT S3-D2	-14.9	11.5	-1.30	0.195	3.28
Spring HT+HS C-D1	-17.4	11.5	-1.51	0.131	3.28
Spring HT+HS C-D2	-8.6	11.5	-0.75	0.454	3.28
Spring HT+HS S1-D1	-3.2	11.5	-0.28	0.780	3.28

Spring HT+HS S1-D2	37.4	11.5	3.24	0.001	3.28
Spring HT+HS S2-D1	-8.9	11.5	-0.78	0.438	3.28
Spring HT+HS S2-D2	4.0	11.5	0.35	0.728	3.28
Spring HT+HS S3-D1	-10.8	11.5	-0.94	0.347	3.28
Summer HS+HT C-D1	-38.4	14.4	-2.66	0.008	3.87
Summer HS+HT C-D2	14.6	14.4	1.01	0.312	3.87
Summer HS+HT S1-D1	-31.4	14.4	-2.17	0.030	3.87
Summer HS+HT S1-D2	46.2	14.4	3.20	0.001	3.87
Summer HS+HT S2-D1	-32.3	14.4	-2.24	0.026	3.87
Summer HS+HT S2-D2	45.8	14.4	3.17	0.002	3.87
Summer HS+HT S3-D1	-33.9	14.4	-2.34	0.019	3.87
Summer HS+HT S3-D2	16.9	14.4	1.17	0.243	3.87
Summer HT+HS C-D1	-37.4	14.4	-2.59	0.010	3.87
Summer HT+HS C-D2	93.0	14.4	6.44	0.000	3.87
Summer HT+HS S1-D1	-35.1	14.4	-2.43	0.015	3.87

Summer HT+HS S1-D2	39.7	14.4	2.75	0.006	3.87
Summer HT+HS S2-D1	-32.3	14.4	-2.23	0.026	3.87
Summer HT+HS S2-D2	35.4	14.4	2.45	0.015	3.87
Summer HT+HS S3-D1	-33.5	14.4	-2.32	0.021	3.87

Table A 3: General Linear Model using Minitab 17: DS% versus Seasons and treatment

Method

Factor coding	(-1, 0, +1)
Rows unused	8

Factor Information

Factor	Type	Levels	Values
Seasons	Fixed	4	Autumn, Spring, Summer, Winter
Treatment	Fixed	16	HS+HT C-D1, HS+HT C-D2, HS+HT S1-D1, HS+HT S1-D2, HS+HT S2-D1, HS+HT S2-D2, HS+HT S3-D1, HS+HT S3-D2, HT+HS C-D1, HT+HS C-D2, HT+HS S1-D1, HT+HS S1-D2, HT+HS S2-D1, HT+HS S2-D2, HT+HS S3-D1, HT+HS S3-D2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Seasons	3	19.445	6.4817	33.61	0.000
Treatment	15	8.793	0.5862	3.04	0.000

Seasons*Treatment	45	27.915	0.6203	3.22	0.000
Error	504	97.190	0.1928		
Total	567	151.250			

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.5863	0.0195	30.07	0.000	
Seasons					
Autumn	0.3441	0.0372	9.25	0.000	1.91
Spring	-0.1956	0.0297	-6.59	0.000	1.72
Summer	-0.1199	0.0372	-3.22	0.001	1.91
Treatment					
HS+HT C-D1	-0.1649	0.0752	-2.19	0.029	2.11
HS+HT C-D2	0.0146	0.0763	0.19	0.849	2.12
HS+HT S1-D1	-0.1627	0.0752	-2.16	0.031	2.11
HS+HT S1-D2	0.0398	0.0752	0.53	0.597	2.11
HS+HT S2-D1	-0.1723	0.0752	-2.29	0.022	2.11
HS+HT S2-D2	-0.1296	0.0752	-1.72	0.085	2.11
HS+HT S3-D1	-0.1440	0.0752	-1.92	0.056	2.11
HS+HT S3-D2	0.1910	0.0752	2.54	0.011	2.11
HT+HS C-D1	0.0435	0.0752	0.58	0.563	2.11
HT+HS C-D2	0.1690	0.0781	2.16	0.031	2.15
HT+HS S1-D1	0.0002	0.0752	0.00	0.998	2.11
HT+HS S1-D2	-0.0356	0.0763	-0.47	0.641	2.12
HT+HS S2-D1	0.0109	0.0752	0.15	0.885	2.11
HT+HS S2-D2	0.0370	0.0752	0.49	0.623	2.11
HT+HS S3-D1	0.0125	0.0752	0.17	0.868	2.11
Seasons*Treatment					
Autumn	-0.304	0.144	-2.11	0.035	3.87
HS+HT C-D1					

Autumn HS+HT C-D2	0.207	0.145	1.43	0.153	3.68
Autumn HS+HT S1-D1	-0.328	0.144	-2.28	0.023	3.87
Autumn HS+HT S1-D2	0.260	0.144	1.81	0.072	3.87
Autumn HS+HT S2-D1	-0.250	0.144	-1.74	0.083	3.87
Autumn HS+HT S2-D2	-0.094	0.144	-0.65	0.513	3.87
Autumn HS+HT S3-D1	-0.290	0.144	-2.01	0.045	3.87
Autumn HS+HT S3-D2	0.764	0.144	5.31	0.000	3.87
Autumn HT+HS C-D1	-0.276	0.144	-1.91	0.056	3.87
Autumn HT+HS C-D2	0.641	0.145	4.40	0.000	3.51
Autumn HT+HS S1-D1	-0.527	0.144	-3.66	0.000	3.87
Autumn HT+HS S1-D2	0.030	0.145	0.21	0.835	3.68
Autumn HT+HS S2-D1	-0.411	0.144	-2.86	0.004	3.87
Autumn HT+HS S2-D2	-0.089	0.144	-0.62	0.536	3.87
Autumn HT+HS S3-D1	-0.188	0.144	-1.31	0.192	3.87
Spring HS+HT C-D1	0.113	0.115	0.98	0.327	3.28

Spring HS+HT C-D2	-0.032	0.116	-0.27	0.785	3.19
Spring HS+HT S1-D1	0.087	0.115	0.76	0.450	3.28
Spring HS+HT S1-D2	-0.087	0.115	-0.76	0.450	3.28
Spring HS+HT S2-D1	0.095	0.115	0.82	0.411	3.28
Spring HS+HT S2-D2	0.104	0.115	0.90	0.368	3.28
Spring HS+HT S3-D1	0.082	0.115	0.72	0.475	3.28
Spring HS+HT S3-D2	-0.228	0.115	-1.99	0.047	3.28
Spring HT+HS C-D1	0.173	0.115	1.51	0.132	3.28
Spring HT+HS C-D2	-0.213	0.117	-1.83	0.068	3.11
Spring HT+HS S1-D1	0.150	0.115	1.31	0.191	3.28
Spring HT+HS S1-D2	-0.072	0.116	-0.63	0.532	3.19
Spring HT+HS S2-D1	0.140	0.115	1.22	0.225	3.28
Spring HT+HS S2-D2	-0.092	0.115	-0.80	0.422	3.28
Spring HT+HS S3-D1	0.090	0.115	0.78	0.434	3.28
Summer HS+HT C-D1	0.188	0.144	1.31	0.191	3.87

Summer HS+HT C-D2	-0.112	0.145	-0.78	0.437	3.68
Summer HS+HT S1-D1	0.219	0.144	1.52	0.128	3.87
Summer HS+HT S1-D2	-0.203	0.144	-1.41	0.159	3.87
Summer HS+HT S2-D1	0.205	0.144	1.43	0.154	3.87
Summer HS+HT S2-D2	-0.030	0.144	-0.21	0.835	3.87
Summer HS+HT S3-D1	0.179	0.144	1.24	0.214	3.87
Summer HS+HT S3-D2	-0.344	0.144	-2.39	0.017	3.87
Summer HT+HS C-D1	0.222	0.144	1.54	0.124	3.87
Summer HT+HS C-D2	-0.284	0.145	-1.95	0.051	3.51
Summer HT+HS S1-D1	0.187	0.144	1.30	0.195	3.87
Summer HT+HS S1-D2	-0.062	0.145	-0.43	0.668	3.68
Summer HT+HS S2-D1	0.230	0.144	1.60	0.111	3.87
Summer HT+HS S2-D2	-0.187	0.144	-1.30	0.195	3.87
Summer HT+HS S3-D1	0.172	0.144	1.20	0.232	3.87

Table A 4: General Linear Model using Minitab 17:-D (3,2) versus Seasons, Treatment Method

Factor coding (-1, 0, +1)

Rows unused 22

Factor Information

Factor	Type	Levels	Values
Seasons	Fixed	4	Autumn, Spring, Summer, Winter
Treatment	Fixed	16	HS+HT C-D1, HS+HT C-D2, HS+HT S1-D1, HS+HT S1-D2, HS+HT S2-D1, HS+HT S2-D2, HS+HT S3-D1, HS+HT S3-D2, HT+HS C-D1, HT+HS C-D2, HT+HS S1-D1, HT+HS S1-D2, HT+HS S2-D1, HT+HS S2-D2, HT+HS S3-D1, HT+HS S3-D2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Seasons	3	0.04884	0.016282	80.56	0.000
Treatment	15	0.03903	0.002602	12.87	0.000
Seasons*Treatment	45	0.03246	0.000721	3.57	0.000
Error	490	0.09904	0.000202		
Total	553	0.21162			

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	0.125670	0.000651	193.01	0.000	
Seasons					
Autumn	0.02008	0.00130	15.46	0.000	2.05
Spring	-0.007045	0.000975	-7.23	0.000	1.78
Summer	-0.00741	0.00122	-6.10	0.000	1.95
Treatment					

HS+HT C-D1	-0.00734	0.00251	-2.92	0.004	2.19
HS+HT C-D2	-0.00806	0.00255	-3.16	0.002	2.19
HS+HT S1-D1	-0.00528	0.00251	-2.10	0.036	2.19
HS+HT S1-D2	-0.00279	0.00251	-1.11	0.267	2.19
HS+HT S2-D1	-0.00172	0.00251	-0.69	0.493	2.19
HS+HT S2-D2	-0.00053	0.00251	-0.21	0.834	2.19
HS+HT S3-D1	-0.00812	0.00251	-3.23	0.001	2.19
HS+HT S3-D2	0.01553	0.00251	6.18	0.000	2.19
HT+HS C-D1	-0.00334	0.00251	-1.33	0.185	2.19
HT+HS C-D2	-0.00469	0.00260	-1.80	0.072	2.21
HT+HS S1-D1	-0.00255	0.00251	-1.02	0.310	2.19
HT+HS S1-D2	-0.00198	0.00251	-0.79	0.431	2.19
HT+HS S2-D1	-0.00179	0.00251	-0.71	0.476	2.19
HT+HS S2-D2	0.00038	0.00251	0.15	0.878	2.19
HT+HS S3-D1	0.00449	0.00251	1.79	0.074	2.19
Seasons*Treatment					
Autumn	-0.01241	0.00503	-2.47	0.014	4.25
HS+HT C-D1					
Autumn	-0.00509	0.00504	-1.01	0.313	4.03
HS+HT C-D2					
Autumn	-0.01407	0.00503	-2.80	0.005	4.25
HS+HT S1-D1					
Autumn	-0.00296	0.00503	-0.59	0.557	4.25
HS+HT S1-D2					
Autumn	-0.00883	0.00503	-1.76	0.080	4.25
HS+HT S2-D1					
Autumn	0.00518	0.00503	1.03	0.303	4.25
HS+HT S2-D2					
Autumn	-0.01003	0.00503	-1.99	0.047	4.25
HS+HT S3-D1					

Autumn HS+HT S3-D2	0.03292	0.00503	6.55	0.000	4.25
Autumn HT+HS C-D1	-0.01101	0.00503	-2.19	0.029	4.25
Autumn HT+HS C-D2	-0.00506	0.00507	-1.00	0.319	3.82
Autumn HT+HS S1-D1	-0.00840	0.00503	-1.67	0.095	4.25
Autumn HT+HS S1-D2	-0.00077	0.00503	-0.15	0.879	4.25
Autumn HT+HS S2-D1	-0.00496	0.00503	-0.99	0.325	4.25
Autumn HT+HS S2-D2	0.00567	0.00503	1.13	0.260	4.25
Autumn HT+HS S3-D1	0.00016	0.00503	0.03	0.975	4.25
Spring HS+HT C-D1	0.00338	0.00377	0.90	0.371	3.38
Spring HS+HT C-D2	0.00302	0.00379	0.79	0.427	3.28
Spring HS+HT S1-D1	0.00349	0.00377	0.92	0.356	3.38
Spring HS+HT S1-D2	0.00100	0.00377	0.27	0.790	3.38
Spring HS+HT S2-D1	0.00077	0.00377	0.20	0.839	3.38
Spring HS+HT S2-D2	-0.00235	0.00377	-0.62	0.534	3.38
Spring HS+HT S3-D1	0.00400	0.00377	1.06	0.289	3.38

Spring HS+HT S3-D2	-0.00715	0.00377	-1.90	0.058	3.38
Spring HT+HS C-D1	0.00322	0.00377	0.85	0.394	3.38
Spring HT+HS C-D2	0.00382	0.00383	1.00	0.320	3.19
Spring HT+HS S1-D1	0.00568	0.00377	1.51	0.133	3.38
Spring HT+HS S1-D2	-0.00123	0.00377	-0.33	0.745	3.38
Spring HT+HS S2-D1	0.00125	0.00377	0.33	0.740	3.38
Spring HT+HS S2-D2	-0.00134	0.00377	-0.36	0.722	3.38
Spring HT+HS S3-D1	0.00380	0.00377	1.01	0.314	3.38
Summer HS+HT C-D1	0.00508	0.00470	1.08	0.281	3.94
Summer HS+HT C-D2	0.00096	0.00472	0.20	0.838	3.75
Summer HS+HT S1-D1	0.00802	0.00470	1.71	0.089	3.94
Summer HS+HT S1-D2	0.00337	0.00470	0.72	0.474	3.94
Summer HS+HT S2-D1	0.00563	0.00470	1.20	0.232	3.94
Summer HS+HT S2-D2	-0.00157	0.00470	-0.33	0.739	3.94
Summer HS+HT S3-D1	0.00203	0.00470	0.43	0.666	3.94

Summer HS+HT S3-D2	-0.02562	0.00470	-5.45	0.000	3.94
Summer HT+HS C-D1	0.00741	0.00470	1.58	0.116	3.94
Summer HT+HS C-D2	0.00160	0.00475	0.34	0.737	3.57
Summer HT+HS S1-D1	0.00346	0.00470	0.74	0.462	3.94
Summer HT+HS S1-D2	-0.00011	0.00470	-0.02	0.981	3.94
Summer HT+HS S2-D1	0.00620	0.00470	1.32	0.188	3.94
Summer HT+HS S2-D2	0.00035	0.00470	0.08	0.940	3.94
Summer HT+HS S3-D1	0.00108	0.00470	0.23	0.818	3.94

Table A 5: -General Linear Model using Minitab 17: - D (4,3) versus Seasons, Treatment Method

Factor coding	(-1, 0, +1)
Rows unused	22

Factor Information

Factor	Type	Levels	Values
Seasons	Fixed	4	Autumn, Spring, Summer, Winter
Treatment	Fixed	16	HS+HT C-D1, HS+HT C-D2, HS+HT S1-D1, HS+HT S1-D2, HS+HT S2-D1, HS+HT S2-D2, HS+HT S3-D1, HS+HT S3-D2, HT+HS C-D1, HT+HS C-D2, HT+HS S1-D1, HT+HS S1-D2, HT+HS S2-D1, HT+HS S2-D2, HT+HS

S3-D1,

HT+HS S3-D2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Seasons	3	237.3	79.100	20.11	0.000
Treatment	15	338.0	22.531	5.73	0.000
Seasons*Treatment	45	304.2	6.760	1.72	0.003
Error	490	1927.1	3.933		
Total	553	2781.6			

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	1.2295	0.0908	13.54	0.000	
Seasons					
Autumn	1.393	0.181	7.69	0.000	2.05
Spring	-0.511	0.136	-3.76	0.000	1.78
Summer	-0.583	0.170	-3.44	0.001	1.95
Treatment					
HS+HT C-D1	-0.975	0.351	-2.78	0.006	2.19
HS+HT C-D2	-0.444	0.356	-1.25	0.212	2.19
HS+HT S1-D1	-0.913	0.351	-2.60	0.009	2.19
HS+HT S1-D2	-0.501	0.351	-1.43	0.154	2.19
HS+HT S2-D1	-0.776	0.351	-2.21	0.027	2.19
HS+HT S2-D2	-0.581	0.351	-1.66	0.098	2.19
HS+HT S3-D1	-0.895	0.351	-2.55	0.011	2.19
HS+HT S3-D2	1.032	0.351	2.94	0.003	2.19
HT+HS C-D1	0.425	0.351	1.21	0.226	2.19
HT+HS C-D2	-0.452	0.363	-1.24	0.214	2.21
HT+HS S1-D1	1.300	0.351	3.71	0.000	2.19

HT+HS S1-D2	1.047	0.351	2.99	0.003	2.19
HT+HS S2-D1	-0.207	0.351	-0.59	0.556	2.19
HT+HS S2-D2	-0.343	0.351	-0.98	0.329	2.19
HT+HS S3-D1	0.749	0.351	2.14	0.033	2.19
Seasons*Treatment					
Autumn	-1.214	0.701	-1.73	0.084	4.25
HS+HT C-D1					
Autumn	0.340	0.704	0.48	0.629	4.03
HS+HT C-D2					
Autumn	-1.118	0.701	-1.59	0.111	4.25
HS+HT S1-D1					
Autumn	-0.206	0.701	-0.29	0.769	4.25
HS+HT S1-D2					
Autumn	-0.714	0.701	-1.02	0.309	4.25
HS+HT S2-D1					
Autumn	-0.457	0.701	-0.65	0.515	4.25
HS+HT S2-D2					
Autumn	-1.233	0.701	-1.76	0.079	4.25
HS+HT S3-D1					
Autumn	0.860	0.701	1.23	0.220	4.25
HS+HT S3-D2					
Autumn	-0.570	0.701	-0.81	0.417	4.25
HT+HS C-D1					
Autumn	-0.788	0.708	-1.11	0.266	3.82
HT+HS C-D2					
Autumn	2.657	0.701	3.79	0.000	4.25
HT+HS S1-D1					
Autumn	4.053	0.701	5.78	0.000	4.25
HT+HS S1-D2					

Autumn HT+HS S2-D1	-0.950	0.701	-1.36	0.176	4.25
Autumn HT+HS S2-D2	-0.454	0.701	-0.65	0.518	4.25
Autumn HT+HS S3-D1	-0.603	0.701	-0.86	0.390	4.25
Spring HS+HT C-D1	0.430	0.526	0.82	0.414	3.38
Spring HS+HT C-D2	-0.083	0.529	-0.16	0.875	3.28
Spring HS+HT S1-D1	0.386	0.526	0.73	0.463	3.38
Spring HS+HT S1-D2	0.011	0.526	0.02	0.983	3.38
Spring HS+HT S2-D1	0.257	0.526	0.49	0.626	3.38
Spring HS+HT S2-D2	0.115	0.526	0.22	0.827	3.38
Spring HS+HT S3-D1	0.417	0.526	0.79	0.428	3.38
Spring HS+HT S3-D2	-0.009	0.526	-0.02	0.986	3.38
Spring HT+HS C-D1	0.282	0.526	0.54	0.592	3.38
Spring HT+HS C-D2	0.192	0.534	0.36	0.719	3.19
Spring HT+HS S1-D1	-0.507	0.526	-0.96	0.335	3.38
Spring HT+HS S1-D2	-1.450	0.526	-2.76	0.006	3.38

Spring HT+HS S2-D1	0.395	0.526	0.75	0.453	3.38
Spring HT+HS S2-D2	-0.046	0.526	-0.09	0.930	3.38
Spring HT+HS S3-D1	0.364	0.526	0.69	0.489	3.38
Summer HS+HT C-D1	0.520	0.656	0.79	0.428	3.94
Summer HS+HT C-D2	-0.009	0.659	-0.01	0.990	3.75
Summer HS+HT S1-D1	0.472	0.656	0.72	0.472	3.94
Summer HS+HT S1-D2	0.183	0.656	0.28	0.781	3.94
Summer HS+HT S2-D1	0.358	0.656	0.55	0.585	3.94
Summer HS+HT S2-D2	0.163	0.656	0.25	0.804	3.94
Summer HS+HT S3-D1	0.443	0.656	0.68	0.500	3.94
Summer HS+HT S3-D2	-1.314	0.656	-2.00	0.046	3.94
Summer HT+HS C-D1	0.690	0.656	1.05	0.293	3.94
Summer HT+HS C-D2	0.310	0.663	0.47	0.640	3.57
Summer HT+HS S1-D1	-0.699	0.656	-1.07	0.287	3.94
Summer HT+HS S1-D2	-1.389	0.656	-2.12	0.035	3.94

Summer HT+HS S2-D1	0.751	0.656	1.15	0.253	3.94
Summer HT+HS S2-D2	0.291	0.656	0.44	0.658	3.94
Summer HT+HS S3-D1	0.216	0.656	0.33	0.742	3.94

Table A 6: General Linear Model using Minitab 17: - L versus Seasons, Treatment

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Seasons	Fixed	4	Autumn, Spring, Summer, Winter
Treatment	Fixed	16	HS+HT C-D1, HS+HT C-D2, HS+HT S1-D1, HS+HT S1-D2, HS+HT S2-D1, HS+HT S2-D2, HS+HT S3-D1, HS+HT S3-D2, HT+HS C-D1, HT+HS C-D2, HT+HS S1-D1, HT+HS S1-D2, HT+HS S2-D1, HT+HS S2-D2, HT+HS S3-D1, HT+HS S3-D2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Seasons	3	12048	4016.14	71.56	0.000
Treatment	15	7848	523.18	9.32	0.000
Seasons*Treatment	45	3583	79.63	1.42	0.042
Error	512	28734	56.12		
Total	575	53467			

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	90.905	0.331	274.57	0.000	
Seasons					
Autumn	-7.207	0.634	-11.37	0.000	1.95
Spring	0.238	0.506	0.47	0.637	1.75
Summer	0.635	0.634	1.00	0.317	1.95
Treatment					
HS+HT C-D1	5.48	1.28	4.28	0.000	2.11
HS+HT C-D2	-1.06	1.28	-0.82	0.411	2.11
HS+HT S1-D1	3.77	1.28	2.94	0.003	2.11
HS+HT S1-D2	-2.53	1.28	-1.97	0.049	2.11
HS+HT S2-D1	2.22	1.28	1.73	0.084	2.11
HS+HT S2-D2	-4.21	1.28	-3.29	0.001	2.11
HS+HT S3-D1	4.13	1.28	3.22	0.001	2.11
HS+HT S3-D2	-5.94	1.28	-4.63	0.000	2.11
HT+HS C-D1	5.45	1.28	4.25	0.000	2.11
HT+HS C-D2	-1.96	1.28	-1.53	0.128	2.11
HT+HS S1-D1	3.42	1.28	2.67	0.008	2.11
HT+HS S1-D2	-2.65	1.28	-2.07	0.039	2.11
HT+HS S2-D1	1.13	1.28	0.88	0.379	2.11
HT+HS S2-D2	-3.95	1.28	-3.08	0.002	2.11
HT+HS S3-D1	3.03	1.28	2.36	0.019	2.11
Seasons*Treatment					
Autumn	4.08	2.46	1.66	0.097	3.87
HS+HT C-D1					
Autumn	-2.33	2.46	-0.95	0.342	3.87
HS+HT C-D2					
Autumn	2.89	2.46	1.18	0.240	3.87
HS+HT S1-D1					

Autumn HS+HT S1-D2	-8.37	2.46	-3.41	0.001	3.87
Autumn HS+HT S2-D1	2.60	2.46	1.06	0.290	3.87
Autumn HS+HT S2-D2	-4.76	2.46	-1.94	0.053	3.87
Autumn HS+HT S3-D1	3.56	2.46	1.45	0.148	3.87
Autumn HS+HT S3-D2	-2.62	2.46	-1.07	0.286	3.87
Autumn HT+HS C-D1	4.62	2.46	1.88	0.060	3.87
Autumn HT+HS C-D2	-4.76	2.46	-1.94	0.053	3.87
Autumn HT+HS S1-D1	5.06	2.46	2.06	0.040	3.87
Autumn HT+HS S1-D2	-4.38	2.46	-1.78	0.075	3.87
Autumn HT+HS S2-D1	1.71	2.46	0.70	0.486	3.87
Autumn HT+HS S2-D2	-2.12	2.46	-0.86	0.389	3.87
Autumn HT+HS S3-D1	5.24	2.46	2.14	0.033	3.87
Spring HS+HT C-D1	0.06	1.96	0.03	0.974	3.28
Spring HS+HT C-D2	0.68	1.96	0.35	0.729	3.28
Spring HS+HT S1-D1	0.22	1.96	0.11	0.910	3.28

Spring HS+HT S1-D2	1.61	1.96	0.82	0.413	3.28
Spring HS+HT S2-D1	0.86	1.96	0.44	0.660	3.28
Spring HS+HT S2-D2	0.18	1.96	0.09	0.928	3.28
Spring HS+HT S3-D1	-0.04	1.96	-0.02	0.984	3.28
Spring HS+HT S3-D2	-0.87	1.96	-0.44	0.658	3.28
Spring HT+HS C-D1	-1.82	1.96	-0.93	0.354	3.28
Spring HT+HS C-D2	1.84	1.96	0.94	0.347	3.28
Spring HT+HS S1-D1	-1.26	1.96	-0.64	0.522	3.28
Spring HT+HS S1-D2	-0.03	1.96	-0.01	0.989	3.28
Spring HT+HS S2-D1	0.23	1.96	0.12	0.908	3.28
Spring HT+HS S2-D2	-0.62	1.96	-0.32	0.750	3.28
Spring HT+HS S3-D1	-1.51	1.96	-0.77	0.441	3.28
Summer HS+HT C-D1	-3.98	2.46	-1.62	0.106	3.87
Summer HS+HT C-D2	2.23	2.46	0.91	0.363	3.87
Summer HS+HT S1-D1	-3.30	2.46	-1.34	0.180	3.87

Summer HS+HT S1-D2	5.83	2.46	2.38	0.018	3.87
Summer HS+HT S2-D1	-4.43	2.46	-1.81	0.072	3.87
Summer HS+HT S2-D2	4.50	2.46	1.83	0.067	3.87
Summer HS+HT S3-D1	-3.84	2.46	-1.57	0.118	3.87
Summer HS+HT S3-D2	5.39	2.46	2.20	0.029	3.87
Summer HT+HS C-D1	-3.41	2.46	-1.39	0.166	3.87
Summer HT+HS C-D2	2.12	2.46	0.86	0.388	3.87
Summer HT+HS S1-D1	-3.99	2.46	-1.62	0.105	3.87
Summer HT+HS S1-D2	5.55	2.46	2.26	0.024	3.87
Summer HT+HS S2-D1	-3.45	2.46	-1.40	0.161	3.87
Summer HT+HS S2-D2	2.55	2.46	1.04	0.300	3.87
Summer HT+HS S3-D1	-4.60	2.46	-1.87	0.062	3.87

Table A 7: General Linear Model using Minitab 17: -: a versus Seasons, Treatment Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values

Seasons	Fixed	4	Autumn, Spring, Summer, Winter
Treatment	Fixed	16	HS+HT C-D1, HS+HT C-D2, HS+HT S1-D1, HS+HT S1-D2, HS+HT S2-D1, HS+HT S2-D2, HS+HT S3-D1, HS+HT S3-D2, HT+HS C-D1, HT+HS C-D2, HT+HS S1-D1, HT+HS S1-D2, HT+HS S2-D1, HT+HS S2-D2, HT+HS S3-D1, HT+HS S3-D2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Seasons	3	1266.5	422.162	68.28	0.000
Treatment	15	182.1	12.139	1.96	0.016
Seasons*Treatment	45	796.8	17.707	2.86	0.000
Error	512	3165.6	6.183		
Total	575	5426.6			

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	-6.175	0.110	-56.19	0.000	
Seasons					
Autumn	-1.033	0.210	-4.91	0.000	1.95
Spring	-0.521	0.168	-3.10	0.002	1.75
Summer	-0.840	0.210	-3.99	0.000	1.95
Treatment					
HS+HT C-D1	0.528	0.426	1.24	0.216	2.11
HS+HT C-D2	0.978	0.426	2.30	0.022	2.11
HS+HT S1-D1	-0.255	0.426	-0.60	0.549	2.11
HS+HT S1-D2	0.227	0.426	0.53	0.595	2.11

HS+HT S2-D1	-0.653	0.426	-1.53	0.126	2.11
HS+HT S2-D2	0.341	0.426	0.80	0.424	2.11
HS+HT S3-D1	-0.365	0.426	-0.86	0.391	2.11
HS+HT S3-D2	-0.541	0.426	-1.27	0.205	2.11
HT+HS C-D1	0.470	0.426	1.10	0.270	2.11
HT+HS C-D2	0.974	0.426	2.29	0.023	2.11
HT+HS S1-D1	-0.190	0.426	-0.45	0.655	2.11
HT+HS S1-D2	0.435	0.426	1.02	0.307	2.11
HT+HS S2-D1	-0.928	0.426	-2.18	0.030	2.11
HT+HS S2-D2	0.343	0.426	0.81	0.420	2.11
HT+HS S3-D1	-0.497	0.426	-1.17	0.244	2.11
Seasons*Treatment					
Autumn	-0.008	0.815	-0.01	0.992	3.87
HS+HT C-D1					
Autumn	-0.657	0.815	-0.81	0.420	3.87
HS+HT C-D2					
Autumn	0.318	0.815	0.39	0.696	3.87
HS+HT S1-D1					
Autumn	-0.679	0.815	-0.83	0.405	3.87
HS+HT S1-D2					
Autumn	-0.181	0.815	-0.22	0.824	3.87
HS+HT S2-D1					
Autumn	-0.253	0.815	-0.31	0.756	3.87
HS+HT S2-D2					
Autumn	0.203	0.815	0.25	0.803	3.87
HS+HT S3-D1					
Autumn	0.432	0.815	0.53	0.597	3.87
HS+HT S3-D2					
Autumn	0.158	0.815	0.19	0.847	3.87
HT+HS C-D1					

Autumn HT+HS C-D2	-0.774	0.815	-0.95	0.342	3.87
Autumn HT+HS S1-D1	0.798	0.815	0.98	0.328	3.87
Autumn HT+HS S1-D2	-0.671	0.815	-0.82	0.411	3.87
Autumn HT+HS S2-D1	-0.145	0.815	-0.18	0.859	3.87
Autumn HT+HS S2-D2	0.366	0.815	0.45	0.653	3.87
Autumn HT+HS S3-D1	0.546	0.815	0.67	0.503	3.87
Spring HS+HT C-D1	-0.618	0.650	-0.95	0.343	3.28
Spring HS+HT C-D2	0.327	0.650	0.50	0.615	3.28
Spring HS+HT S1-D1	-0.625	0.650	-0.96	0.337	3.28
Spring HS+HT S1-D2	1.249	0.650	1.92	0.055	3.28
Spring HS+HT S2-D1	-0.308	0.650	-0.47	0.636	3.28
Spring HS+HT S2-D2	0.279	0.650	0.43	0.668	3.28
Spring HS+HT S3-D1	-0.813	0.650	-1.25	0.212	3.28
Spring HS+HT S3-D2	0.843	0.650	1.30	0.195	3.28
Spring HT+HS C-D1	-0.657	0.650	-1.01	0.312	3.28

Spring HT+HS C-D2	0.692	0.650	1.06	0.287	3.28
Spring HT+HS S1-D1	-1.050	0.650	-1.62	0.107	3.28
Spring HT+HS S1-D2	0.444	0.650	0.68	0.495	3.28
Spring HT+HS S2-D1	-0.580	0.650	-0.89	0.373	3.28
Spring HT+HS S2-D2	0.791	0.650	1.22	0.224	3.28
Spring HT+HS S3-D1	-1.001	0.650	-1.54	0.124	3.28
Summer HS+HT C-D1	-1.135	0.815	-1.39	0.164	3.87
Summer HS+HT C-D2	1.713	0.815	2.10	0.036	3.87
Summer HS+HT S1-D1	-1.028	0.815	-1.26	0.208	3.87
Summer HS+HT S1-D2	1.048	0.815	1.29	0.199	3.87
Summer HS+HT S2-D1	-0.847	0.815	-1.04	0.299	3.87
Summer HS+HT S2-D2	1.686	0.815	2.07	0.039	3.87
Summer HS+HT S3-D1	-1.230	0.815	-1.51	0.132	3.87
Summer HS+HT S3-D2	1.179	0.815	1.45	0.149	3.87
Summer HT+HS C-D1	-1.357	0.815	-1.66	0.097	3.87

Summer HT+HS C-D2	0.948	0.815	1.16	0.245	3.87
Summer HT+HS S1-D1	-1.350	0.815	-1.66	0.098	3.87
Summer HT+HS S1-D2	1.323	0.815	1.62	0.105	3.87
Summer HT+HS S2-D1	-0.994	0.815	-1.22	0.223	3.87
Summer HT+HS S2-D2	0.540	0.815	0.66	0.508	3.87
Summer HT+HS S3-D1	-1.222	0.815	-1.50	0.134	3.87

Table A 8: General Linear Model using Minitab 17:-b versus Seasons, Treatment

Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Seasons	Fixed	4	Autumn, Spring, Summer, Winter
Treatment	Fixed	16	HS+HT C-D1, HS+HT C-D2, HS+HT S1-D1, HS+HT S1-D2, HS+HT S2-D1, HS+HT S2-D2, HS+HT S3-D1, HS+HT S3-D2, HT+HS C-D1, HT+HS

C-D2,

HT+HS S1-D1, HT+HS S1-D2, HT+HS S2-D1, HT+HS S2-D2, HT+HS S3-D1,

HT+HS S3-D2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Seasons	3	7805	2601.61	156.05	0.000
Treatment	15	2166	144.39	8.66	0.000
Seasons*Treatment	45	1937	43.05	2.58	0.000
Error	512	8536	16.67		
Total	575	20822			

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	8.400	0.180	46.55	0.000	
Seasons					
Autumn	6.229	0.346	18.03	0.000	1.95
Spring	-1.118	0.276	-4.06	0.000	1.75
Summer	-0.332	0.346	-0.96	0.338	1.95
Treatment					
HS+HT C-D1	1.070	0.699	1.53	0.126	2.11
HS+HT C-D2	-1.389	0.699	-1.99	0.047	2.11
HS+HT S1-D1	1.901	0.699	2.72	0.007	2.11
HS+HT S1-D2	-1.430	0.699	-2.05	0.041	2.11
HS+HT S2-D1	2.520	0.699	3.61	0.000	2.11
HS+HT S2-D2	-2.468	0.699	-3.53	0.000	2.11
HS+HT S3-D1	1.856	0.699	2.66	0.008	2.11
HS+HT S3-D2	-2.574	0.699	-3.68	0.000	2.11
HT+HS C-D1	1.416	0.699	2.03	0.043	2.11
HT+HS C-D2	-1.325	0.699	-1.90	0.059	2.11

HT+HS S1-D1	1.840	0.699	2.63	0.009	2.11
HT+HS S1-D2	-1.624	0.699	-2.32	0.021	2.11
HT+HS S2-D1	2.817	0.699	4.03	0.000	2.11
HT+HS S2-D2	-2.918	0.699	-4.18	0.000	2.11
HT+HS S3-D1	2.408	0.699	3.45	0.001	2.11
Seasons*Treatment					
Autumn	-2.77	1.34	-2.07	0.039	3.87
HS+HT C-D1					
Autumn	2.45	1.34	1.83	0.068	3.87
HS+HT C-D2					
Autumn	-2.78	1.34	-2.08	0.038	3.87
HS+HT S1-D1					
Autumn	2.64	1.34	1.97	0.049	3.87
HS+HT S1-D2					
Autumn	-2.71	1.34	-2.02	0.044	3.87
HS+HT S2-D1					
Autumn	2.76	1.34	2.06	0.040	3.87
HS+HT S2-D2					
Autumn	-3.16	1.34	-2.36	0.019	3.87
HS+HT S3-D1					
Autumn	3.86	1.34	2.88	0.004	3.87
HS+HT S3-D2					
Autumn	-2.39	1.34	-1.79	0.075	3.87
HT+HS C-D1					
Autumn	1.12	1.34	0.83	0.405	3.87
HT+HS C-D2					
Autumn	-3.54	1.34	-2.64	0.008	3.87
HT+HS S1-D1					
Autumn	2.65	1.34	1.98	0.049	3.87
HT+HS S1-D2					

Autumn HT+HS S2-D1	-2.05	1.34	-1.53	0.127	3.87
Autumn HT+HS S2-D2	1.85	1.34	1.39	0.167	3.87
Autumn HT+HS S3-D1	-2.17	1.34	-1.62	0.106	3.87
Spring HS+HT C-D1	1.41	1.07	1.32	0.186	3.28
Spring HS+HT C-D2	-0.17	1.07	-0.16	0.876	3.28
Spring HS+HT S1-D1	1.23	1.07	1.15	0.250	3.28
Spring HS+HT S1-D2	-2.37	1.07	-2.22	0.027	3.28
Spring HS+HT S2-D1	1.09	1.07	1.02	0.310	3.28
Spring HS+HT S2-D2	-0.56	1.07	-0.53	0.598	3.28
Spring HS+HT S3-D1	1.61	1.07	1.51	0.132	3.28
Spring HS+HT S3-D2	-2.46	1.07	-2.31	0.021	3.28
Spring HT+HS C-D1	1.09	1.07	1.02	0.308	3.28
Spring HT+HS C-D2	-0.84	1.07	-0.78	0.433	3.28
Spring HT+HS S1-D1	1.48	1.07	1.39	0.165	3.28
Spring HT+HS S1-D2	-0.80	1.07	-0.75	0.454	3.28

Spring HT+HS S2-D1	0.81	1.07	0.76	0.447	3.28
Spring HT+HS S2-D2	-0.47	1.07	-0.44	0.657	3.28
Spring HT+HS S3-D1	1.22	1.07	1.14	0.254	3.28
Summer HS+HT C-D1	2.48	1.34	1.85	0.065	3.87
Summer HS+HT C-D2	-3.66	1.34	-2.73	0.007	3.87
Summer HS+HT S1-D1	2.17	1.34	1.62	0.106	3.87
Summer HS+HT S1-D2	-1.99	1.34	-1.49	0.138	3.87
Summer HS+HT S2-D1	2.42	1.34	1.81	0.072	3.87
Summer HS+HT S2-D2	-3.12	1.34	-2.33	0.020	3.87
Summer HS+HT S3-D1	2.48	1.34	1.85	0.064	3.87
Summer HS+HT S3-D2	-1.88	1.34	-1.41	0.160	3.87
Summer HT+HS C-D1	2.60	1.34	1.94	0.052	3.87
Summer HT+HS C-D2	-1.39	1.34	-1.04	0.301	3.87
Summer HT+HS S1-D1	2.71	1.34	2.02	0.043	3.87
Summer HT+HS S1-D2	-2.01	1.34	-1.50	0.133	3.87

Summer HT+HS S2-D1	2.45	1.34	1.83	0.067	3.87
Summer HT+HS S2-D2	-2.46	1.34	-1.84	0.067	3.87
Summer HT+HS S3-D1	2.27	1.34	1.70	0.090	3.87

Table A 9: -General Linear Model using Minitab 17: -Hue angle versus Seasons, Treatment Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values
Seasons	Fixed	4	Autumn, Spring, Summer, Winter
Treatment	Fixed	16	HS+HT C-D1, HS+HT C-D2, HS+HT S1-D1, HS+HT S1-D2, HS+HT S2-D1, HS+HT S2-D2, HS+HT S3-D1, HS+HT S3-D2, HT+HS C-D1, HT+HS C-D2, HT+HS S1-D1, HT+HS S1-D2, HT+HS S2-D1, HT+HS S2-D2, HT+HS S3-D1, HT+HS S3-D2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Seasons	3	31860	10619.9	20.57	0.000
Treatment	15	43454	2897.0	5.61	0.000
Seasons*Treatment	45	31956	710.1	1.38	0.058
Error	512	264313	516.2		
Total	575	390812			

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	-48.16	1.00	-47.96	0.000	
Seasons					
Autumn	-14.91	1.92	-7.76	0.000	1.95
Spring	5.45	1.53	3.55	0.000	1.75
Summer	4.70	1.92	2.44	0.015	1.95
Treatment					
HS+HT C-D1	-11.32	3.89	-2.91	0.004	2.11
HS+HT C-D2	-0.72	3.89	-0.19	0.853	2.11
HS+HT S1-D1	-4.56	3.89	-1.17	0.242	2.11
HS+HT S1-D2	3.44	3.89	0.88	0.377	2.11
HS+HT S2-D1	-10.95	3.89	-2.82	0.005	2.11
HS+HT S2-D2	7.77	3.89	2.00	0.046	2.11
HS+HT S3-D1	-5.84	3.89	-1.50	0.134	2.11
HS+HT S3-D2	20.44	3.89	5.26	0.000	2.11
HT+HS C-D1	-5.25	3.89	-1.35	0.178	2.11
HT+HS C-D2	-1.49	3.89	-0.38	0.702	2.11
HT+HS S1-D1	-4.94	3.89	-1.27	0.205	2.11
HT+HS S1-D2	2.38	3.89	0.61	0.540	2.11
HT+HS S2-D1	-10.30	3.89	-2.65	0.008	2.11
HT+HS S2-D2	9.11	3.89	2.34	0.020	2.11
HT+HS S3-D1	-4.87	3.89	-1.25	0.211	2.11
Seasons*Treatment					
Autumn	11.75	7.45	1.58	0.115	3.87
HS+HT C-D1					
Autumn	-1.84	7.45	-0.25	0.805	3.87
HS+HT C-D2					
Autumn	5.25	7.45	0.70	0.481	3.87
HS+HT S1-D1					

Autumn HS+HT S1-D2	-1.76	7.45	-0.24	0.813	3.87
Autumn HS+HT S2-D1	13.05	7.45	1.75	0.080	3.87
Autumn HS+HT S2-D2	-8.40	7.45	-1.13	0.260	3.87
Autumn HS+HT S3-D1	7.65	7.45	1.03	0.305	3.87
Autumn HS+HT S3-D2	-22.95	7.45	-3.08	0.002	3.87
Autumn HT+HS C-D1	4.00	7.45	0.54	0.591	3.87
Autumn HT+HS C-D2	5.49	7.45	0.74	0.461	3.87
Autumn HT+HS S1-D1	5.45	7.45	0.73	0.465	3.87
Autumn HT+HS S1-D2	-2.58	7.45	-0.35	0.729	3.87
Autumn HT+HS S2-D1	11.55	7.45	1.55	0.121	3.87
Autumn HT+HS S2-D2	-10.17	7.45	-1.37	0.173	3.87
Autumn HT+HS S3-D1	3.62	7.45	0.49	0.627	3.87
Spring HS+HT C-D1	-1.23	5.94	-0.21	0.837	3.28
Spring HS+HT C-D2	-2.62	5.94	-0.44	0.660	3.28
Spring HS+HT S1-D1	-4.95	5.94	-0.83	0.405	3.28

Spring HS+HT S1-D2	6.76	5.94	1.14	0.256	3.28
Spring HS+HT S2-D1	-0.51	5.94	-0.09	0.931	3.28
Spring HS+HT S2-D2	1.41	5.94	0.24	0.813	3.28
Spring HS+HT S3-D1	-3.59	5.94	-0.60	0.546	3.28
Spring HS+HT S3-D2	6.00	5.94	1.01	0.313	3.28
Spring HT+HS C-D1	-5.17	5.94	-0.87	0.384	3.28
Spring HT+HS C-D2	0.51	5.94	0.09	0.932	3.28
Spring HT+HS S1-D1	-2.38	5.94	-0.40	0.689	3.28
Spring HT+HS S1-D2	2.10	5.94	0.35	0.724	3.28
Spring HT+HS S2-D1	2.16	5.94	0.36	0.717	3.28
Spring HT+HS S2-D2	0.52	5.94	0.09	0.930	3.28
Spring HT+HS S3-D1	-3.52	5.94	-0.59	0.553	3.28
Summer HS+HT C-D1	-1.95	7.45	-0.26	0.794	3.87
Summer HS+HT C-D2	10.14	7.45	1.36	0.174	3.87
Summer HS+HT S1-D1	-7.59	7.45	-1.02	0.308	3.87

Summer HS+HT S1-D2	2.43	7.45	0.33	0.744	3.87
Summer HS+HT S2-D1	-2.37	7.45	-0.32	0.750	3.87
Summer HS+HT S2-D2	11.34	7.45	1.52	0.128	3.87
Summer HS+HT S3-D1	-5.94	7.45	-0.80	0.426	3.87
Summer HS+HT S3-D2	-5.17	7.45	-0.69	0.488	3.87
Summer HT+HS C-D1	-8.11	7.45	-1.09	0.276	3.87
Summer HT+HS C-D2	0.35	7.45	0.05	0.963	3.87
Summer HT+HS S1-D1	-7.46	7.45	-1.00	0.317	3.87
Summer HT+HS S1-D2	2.06	7.45	0.28	0.783	3.87
Summer HT+HS S2-D1	-2.42	7.45	-0.33	0.745	3.87
Summer HT+HS S2-D2	16.09	7.45	2.16	0.031	3.87
Summer HT+HS S3-D1	-7.26	7.45	-0.98	0.330	3.87

Table A 10: - General Linear Model using Minitab 17: Chroma versus Seasons, Treatment Method

Factor coding (-1, 0, +1)

Factor Information

Factor	Type	Levels	Values

Seasons	Fixed	4	Autumn, Spring, Summer, Winter
Treatment	Fixed	16	HS+HT C-D1, HS+HT C-D2, HS+HT S1-D1, HS+HT S1-D2, HS+HT S2-D1, HS+HT S2-D2, HS+HT S3-D1, HS+HT S3-D2, HT+HS C-D1, HT+HS C-D2, HT+HS S1-D1, HT+HS S1-D2, HT+HS S2-D1, HT+HS S2-D2, HT+HS S3-D1, HT+HS S3-D2

Analysis of Variance

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Seasons	3	6250.5	2083.50	123.14	0.000
Treatment	15	997.2	66.48	3.93	0.000
Seasons*Treatment	45	2872.9	63.84	3.77	0.000
Error	512	8662.6	16.92		
Total	575	18684.8			

Coefficients

Term	Coef	SE Coef	T-Value	P-Value	VIF
Constant	11.014	0.182	60.59	0.000	
Seasons					
Autumn	5.350	0.348	15.37	0.000	1.95
Spring	-0.881	0.278	-3.17	0.002	1.75
Summer	-0.016	0.348	-0.04	0.964	1.95
Treatment					
HS+HT C-D1	0.152	0.704	0.22	0.829	2.11
HS+HT C-D2	-1.562	0.704	-2.22	0.027	2.11
HS+HT S1-D1	1.212	0.704	1.72	0.086	2.11
HS+HT S1-D2	-1.264	0.704	-1.80	0.073	2.11

HS+HT S2-D1	1.910	0.704	2.71	0.007	2.11
HS+HT S2-D2	-1.731	0.704	-2.46	0.014	2.11
HS+HT S3-D1	1.236	0.704	1.76	0.080	2.11
HS+HT S3-D2	-0.919	0.704	-1.31	0.192	2.11
HT+HS C-D1	0.512	0.704	0.73	0.467	2.11
HT+HS C-D2	-1.307	0.704	-1.86	0.064	2.11
HT+HS S1-D1	1.134	0.704	1.61	0.108	2.11
HT+HS S1-D2	-1.364	0.704	-1.94	0.053	2.11
HT+HS S2-D1	2.324	0.704	3.30	0.001	2.11
HT+HS S2-D2	-1.669	0.704	-2.37	0.018	2.11
HT+HS S3-D1	1.767	0.704	2.51	0.012	2.11
Seasons*Treatment					
Autumn	-1.90	1.35	-1.41	0.160	3.87
HS+HT C-D1					
Autumn	2.34	1.35	1.73	0.083	3.87
HS+HT C-D2					
Autumn	-1.96	1.35	-1.45	0.146	3.87
HS+HT S1-D1					
Autumn	2.60	1.35	1.93	0.055	3.87
HS+HT S1-D2					
Autumn	-1.67	1.35	-1.24	0.216	3.87
HS+HT S2-D1					
Autumn	1.91	1.35	1.42	0.158	3.87
HS+HT S2-D2					
Autumn	-2.30	1.35	-1.71	0.089	3.87
HS+HT S3-D1					
Autumn	2.07	1.35	1.54	0.125	3.87
HS+HT S3-D2					
Autumn	-1.71	1.35	-1.27	0.206	3.87
HT+HS C-D1					

Autumn HT+HS C-D2	1.14	1.35	0.85	0.398	3.87
Autumn HT+HS S1-D1	-2.92	1.35	-2.17	0.031	3.87
Autumn HT+HS S1-D2	2.38	1.35	1.76	0.078	3.87
Autumn HT+HS S2-D1	-1.17	1.35	-0.87	0.385	3.87
Autumn HT+HS S2-D2	0.35	1.35	0.26	0.797	3.87
Autumn HT+HS S3-D1	-1.62	1.35	-1.20	0.229	3.87
Spring HS+HT C-D1	1.62	1.08	1.51	0.132	3.28
Spring HS+HT C-D2	-0.68	1.08	-0.63	0.527	3.28
Spring HS+HT S1-D1	1.55	1.08	1.44	0.149	3.28
Spring HS+HT S1-D2	-2.55	1.08	-2.37	0.018	3.28
Spring HS+HT S2-D1	1.29	1.08	1.19	0.233	3.28
Spring HS+HT S2-D2	-0.93	1.08	-0.86	0.389	3.28
Spring HS+HT S3-D1	1.97	1.08	1.83	0.067	3.28
Spring HS+HT S3-D2	-2.06	1.08	-1.92	0.056	3.28
Spring HT+HS C-D1	1.33	1.08	1.24	0.216	3.28

Spring HT+HS C-D2	-1.60	1.08	-1.49	0.138	3.28
Spring HT+HS S1-D1	2.04	1.08	1.89	0.059	3.28
Spring HT+HS S1-D2	-1.13	1.08	-1.05	0.294	3.28
Spring HT+HS S2-D1	1.24	1.08	1.16	0.248	3.28
Spring HT+HS S2-D2	-1.51	1.08	-1.41	0.160	3.28
Spring HT+HS S3-D1	1.79	1.08	1.66	0.097	3.28
Summer HS+HT C-D1	2.74	1.35	2.04	0.042	3.87
Summer HS+HT C-D2	-4.12	1.35	-3.06	0.002	3.87
Summer HS+HT S1-D1	2.49	1.35	1.85	0.065	3.87
Summer HS+HT S1-D2	-2.29	1.35	-1.70	0.091	3.87
Summer HS+HT S2-D1	2.64	1.35	1.96	0.051	3.87
Summer HS+HT S2-D2	-3.59	1.35	-2.66	0.008	3.87
Summer HS+HT S3-D1	2.87	1.35	2.13	0.034	3.87
Summer HS+HT S3-D2	-2.68	1.35	-1.99	0.047	3.87
Summer HT+HS C-D1	2.93	1.35	2.17	0.030	3.87

Summer HT+HS C-D2	-2.23	1.35	-1.66	0.098	3.87
Summer HT+HS S1-D1	3.11	1.35	2.31	0.021	3.87
Summer HT+HS S1-D2	-2.72	1.35	-2.02	0.044	3.87
Summer HT+HS S2-D1	2.73	1.35	2.03	0.043	3.87
Summer HT+HS S2-D2	-1.84	1.35	-1.36	0.173	3.87
Summer HT+HS S3-D1	2.69	1.35	2.00	0.047	3.87

Comparisons for PH

Tukey Pairwise Comparisons: Seasons

Grouping Information Using the Tukey Method and 95% Confidence

Seasons	N	Mean	Grouping			
Autumn	96	6.64500	A			
Winter	192	6.47302		B		
Spring	192	6.35891			C	
Summer	96	6.23646				D

Means that do not share a letter are significantly different.

Fisher Pairwise Comparisons: Seasons

Grouping Information Using Fisher LSD Method and 95% Confidence

Seasons	N	Mean	Grouping		
Autumn	96	6.64500	A		
Winter	192	6.47302		B	
Spring	192	6.35891			C

Summer	96	6.23646	D

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatment

Grouping Information Using the Tukey Method and 95% Confidence

Treatment	N	Mean	Grouping				
HT+HS S2-D1	36	6.91292	A				
HS+HT S2-D1	36	6.88771	A				
HT+HS S1-D1	36	6.80479	A	B			
HS+HT S1-D1	36	6.80292	A	B			
HT+HS C-D1	36	6.63563		B	C		
HS+HT C-D1	36	6.60688		B	C		
HT+HS S3-D1	36	6.46938			C		
HS+HT S3-D1	36	6.43896			C	D	
HS+HT S3-D2	36	6.24417				D	E
HT+HS S3-D2	36	6.22292					E
HT+HS S2-D2	36	6.19792					E
HT+HS S1-D2	36	6.16375					E
HT+HS C-D2	36	6.15875					E
HS+HT S2-D2	36	6.14854					E
HS+HT S1-D2	36	6.08813					E
HS+HT C-D2	36	6.07021					E

Means that do not share a letter are significantly different.

Fisher Pairwise Comparisons: Treatment

Grouping Information Using Fisher LSD Method and 95% Confidence

Treatment	N	Mean	Grouping					
HT+HS S2-D1	36	6.91292	A					
HS+HT S2-D1	36	6.88771	A					
HT+HS S1-D1	36	6.80479	A					
HS+HT S1-D1	36	6.80292	A					
HT+HS C-D1	36	6.63563		B				
HS+HT C-D1	36	6.60688		B				
HT+HS S3-D1	36	6.46938			C			
HS+HT S3-D1	36	6.43896			C			
HS+HT S3-D2	36	6.24417				D		
HT+HS S3-D2	36	6.22292				D		
HT+HS S2-D2	36	6.19792				D	E	
HT+HS S1-D2	36	6.16375				D	E	F
HT+HS C-D2	36	6.15875				D	E	F
HS+HT S2-D2	36	6.14854				D	E	F
HS+HT S1-D2	36	6.08813					E	F
HS+HT C-D2	36	6.07021						F

Tukey Pairwise Comparisons: Seasons*Treatment

Grouping Information Using the Tukey Method and 95% Confidence

Seasons*Treatment	N	Mean	Grouping
Autumn HT+HS S2-D1	6	7.01500	A
Autumn HS+HT S2-D1	6	7.00333	A
Spring HS+HT S2-D1	12	6.89667	A
Spring HT+HS S2-D1	12	6.88167	A B

Winter	12	6.87833	A	B					
HT+HS S2-D1									
Autumn	6	6.87667	A	B	C	D			
HT+HS S1-D1									
Summer	6	6.87667	A	B	C	D			
HT+HS S2-D1									
Autumn	6	6.86833	A	B	C	D			
HS+HT S1-D1									
Summer	6	6.86833	A	B	C	D			
HS+HT S2-D1									
Spring	12	6.81917	A	B	C				
HS+HT S1-D1									
Winter	12	6.79167	A	B	C	D			
HT+HS S1-D1									
Spring	12	6.78750	A	B	C	D			
HT+HS S1-D1									
Winter	12	6.78250	A	B	C	D			
HS+HT S2-D1									
Winter	12	6.77917	A	B	C	D			
HS+HT S1-D1									
Summer	6	6.76333	A	B	C	D	E		
HT+HS S1-D1									
Summer	6	6.74500	A	B	C	D	E	F	
HS+HT S1-D1									
Autumn	6	6.71167	A	B	C	D	E	F	G
HT+HS C-D1									
Autumn	6	6.68833	A	B	C	D	E	F	G H
HT+HS S2-D2									
Autumn	6	6.68000	A	B	C	D	E	F	G H
HS+HT C-D1									

Autumn	6	6.67167	A	B	C	D	E	F	G	H			
HS+HT S2-D2													
Spring	12	6.62333	A	B	C	D	E	F	G				
HS+HT C-D1													
Spring	12	6.61917	A	B	C	D	E	F	G				
HT+HS S3-D1													
Winter	12	6.61833	A	B	C	D	E	F	G				
HT+HS C-D1													
Spring	12	6.61583	A	B	C	D	E	F	G				
HT+HS C-D1													
Autumn	6	6.59667	A	B	C	D	E	F	G	H	I		
HT+HS S1-D2													
Summer	6	6.59667	A	B	C	D	E	F	G	H	I		
HT+HS C-D1													
Winter	12	6.57417	A	B	C	D	E	F	G	H			
HS+HT C-D1													
Summer	6	6.55000	A	B	C	D	E	F	G	H	I	J	
HS+HT C-D1													
Autumn	6	6.54000	A	B	C	D	E	F	G	H	I	J	
HS+HT S1-D2													
Autumn	6	6.51833	A	B	C	D	E	F	G	H	I	J	K
HS+HT S3-D2													
Autumn	6	6.48167	A	B	C	D	E	F	G	H	I	J	K L
HS+HT S3-D1													
Autumn	6	6.48000	A	B	C	D	E	F	G	H	I	J	K L
HT+HS C-D2													
Autumn	6	6.46333	A	B	C	D	E	F	G	H	I	J	K L M
HT+HS S3-D1													
Winter	12	6.46000			C	D	E	F	G	H	I	J	
HS+HT S3-D1													

Autumn HT+HS S3-D2	6	6.45667	A	B	C	D	E	F	G	H	I	J	K	L	M				
Winter HT+HS S3-D2	12	6.44583			C	D	E	F	G	H	I	J							
Spring HS+HT S3-D1	12	6.43917			C	D	E	F	G	H	I	J	K						
Winter HS+HT S3-D2	12	6.42750			C	D	E	F	G	H	I	J	K						
Winter HT+HS C-D2	12	6.41583			C	D	E	F	G	H	I	J	K	L					
Winter HT+HS S3-D1	12	6.40333				D	E	F	G	H	I	J	K	L					
Summer HT+HS S3-D1	6	6.39167		B	C	D	E	F	G	H	I	J	K	L	M	N			
Summer HS+HT S3-D1	6	6.37500			C	D	E	F	G	H	I	J	K	L	M	N	O		
Winter HT+HS S1-D2	12	6.29667					E	F	G	H	I	J	K	L	M	N	O		
Autumn HS+HT C-D2	6	6.26833					E	F	G	H	I	J	K	L	M	N	O	P	Q
Spring HS+HT S3-D2	12	6.25583						F	G	H	I	J	K	L	M	N	O	P	
Winter HS+HT C-D2	12	6.24167							G	H	I	J	K	L	M	N	O	P	
Winter HT+HS S2-D2	12	6.19917								H	I	J	K	L	M	N	O	P	Q
Winter HS+HT S2-D2	12	6.14333									I	J	K	L	M	N	O	P	Q
Winter HS+HT S1-D2	12	6.11083									I	J	K	L	M	N	O	P	Q

Spring HT+HS S2-D2	12	6.03417			K	L	M	N	O	P	Q
Spring HT+HS S3-D2	12	6.01583				L	M	N	O	P	Q
Spring HT+HS C-D2	12	6.01083				L	M	N	O	P	Q
Spring HS+HT S2-D2	12	5.97750					M	N	O	P	Q
Summer HT+HS S3-D2	6	5.97333		J	K	L	M	N	O	P	Q
Spring HT+HS S1-D2	12	5.93333						N	O	P	Q
Spring HS+HT S1-D2	12	5.92667						N	O	P	Q
Spring HS+HT C-D2	12	5.90583						N	O	P	Q
Summer HT+HS S2-D2	6	5.87000						N	O	P	Q
Summer HS+HT C-D2	6	5.86500						N	O	P	Q
Summer HT+HS S1-D2	6	5.82833						N	O	P	Q
Summer HS+HT S2-D2	6	5.80167							O	P	Q
Summer HS+HT S1-D2	6	5.77500								P	Q
Summer HS+HT S3-D2	6	5.77500								P	Q
Summer HT+HS C-D2	6	5.72833									Q

Comparisons for L

Tukey Pairwise Comparisons: Seasons

Grouping Information Using the Tukey Method and 95% Confidence

Seasons	N	Mean	Grouping		
Winter	192	97.2393	A		
Summer	96	91.5400		B	
Spring	192	91.1436		B	
Autumn	96	83.6978			C

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatment

Grouping Information Using the Tukey Method and 95% Confidence

Treatment	N	Mean	Grouping					
HS+HT C-D1	36	96.3877	A					
HT+HS C-D1	36	96.3554	A					
HS+HT S3-D1	36	95.0390	A	B				
HS+HT S1-D1	36	94.6738	A	B	C			
HT+HS S1-D1	36	94.3294	A	B	C			
HT+HS S3-D1	36	93.9308	A	B	C			
HS+HT S2-D1	36	93.1217	A	B	C	D		
HT+HS S2-D1	36	92.0335	A	B	C	D	E	
HS+HT C-D2	36	89.8496		B	C	D	E	F
HT+HS C-D2	36	88.9490		B	C	D	E	F
HS+HT S1-D2	36	88.3771			C	D	E	F
HT+HS S1-D2	36	88.2517			C	D	E	F
HT+HS S2-D2	36	86.9565				D	E	F
HS+HT S2-D2	36	86.6904					E	F

HS+HT S3-D2	36	84.9671	F
HT+HS S3-D2	36	84.5704	F

Means that do not share a letter are significantly different.

Tukey Pairwise Comparisons: Treatment

Grouping Information Using the Tukey Method and 95% Confidence

Treatment	N	Mean	Grouping					
HS+HT C-D1	36	96.3877	A					
HT+HS C-D1	36	96.3554	A					
HS+HT S3-D1	36	95.0390	A	B				
HS+HT S1-D1	36	94.6738	A	B	C			
HT+HS S1-D1	36	94.3294	A	B	C			
HT+HS S3-D1	36	93.9308	A	B	C			
HS+HT S2-D1	36	93.1217	A	B	C	D		
HT+HS S2-D1	36	92.0335	A	B	C	D	E	
HS+HT C-D2	36	89.8496		B	C	D	E	F
HT+HS C-D2	36	88.9490		B	C	D	E	F
HS+HT S1-D2	36	88.3771			C	D	E	F
HT+HS S1-D2	36	88.2517			C	D	E	F
HT+HS S2-D2	36	86.9565				D	E	F
HS+HT S2-D2	36	86.6904					E	F
HS+HT S3-D2	36	84.9671						F
HT+HS S3-D2	36	84.5704						F

Means that do not share a letter are significantly different.

Grouping Information Using the Tukey Method and 95% Confidence

Seasons*Treatment	N	Mean	Grouping
Winter	12	103.292	A
HT+HS C-D1			

Winter HS+HT C-D1	12	102.558	A	B					
Winter HS+HT S3-D1	12	101.695	A	B	C				
Winter HS+HT S1-D1	12	101.193	A	B	C				
Winter HT+HS S3-D1	12	101.129	A	B	C				
Winter HT+HS S1-D1	12	100.847	A	B	C	D			
Winter HS+HT S2-D1	12	100.428	A	B	C	D	E		
Winter HT+HS S2-D1	12	99.880	A	B	C	D	E		
Spring HS+HT C-D1	12	96.690	A	B	C	D	E	F	
Winter HT+HS C-D2	12	96.073	A	B	C	D	E	F	
Winter HS+HT S1-D2	12	95.648	A	B	C	D	E	F	G
Winter HS+HT C-D2	12	95.605	A	B	C	D	E	F	G
Spring HS+HT S3-D1	12	95.238	A	B	C	D	E	F	G
Spring HS+HT S1-D1	12	95.133	A	B	C	D	E	F	G
Summer HS+HT S1-D2	6	94.845	A	B	C	D	E	F	G H
Spring HT+HS C-D1	12	94.777	A	B	C	D	E	F	G

Summer HT+HS S1-D2	6	94.440	A	B	C	D	E	F	G	H	I			
Spring HS+HT S2-D1	12	94.222	A	B	C	D	E	F	G					
Autumn HT+HS C-D1	6	93.768	A	B	C	D	E	F	G	H	I	J		
Summer HT+HS C-D1	6	93.585	A	B	C	D	E	F	G	H	I	J		
Winter HT+HS S2-D2	12	93.485	A	B	C	D	E	F	G					
Winter HT+HS S1-D2	12	93.439	A	B	C	D	E	F	G					
Spring HT+HS S1-D1	12	93.313	A	B	C	D	E	F	G					
Autumn HS+HT C-D1	6	93.258	A	B	C	D	E	F	G	H	I	J		
Winter HS+HT S2-D2	12	93.103	A	B	C	D	E	F	G					
Summer HS+HT C-D1	6	93.045	A	B	C	D	E	F	G	H	I	J		
Summer HS+HT C-D2	6	92.718	A	B	C	D	E	F	G	H	I	J	K	
Spring HT+HS S3-D1	12	92.657	A	B	C	D	E	F	G	H				
Spring HT+HS S2-D1	12	92.498	A	B	C	D	E	F	G	H				
Autumn HT+HS S1-D1	6	92.180	A	B	C	D	E	F	G	H	I	J	K	L
Summer HS+HT S1-D1	6	92.013	A	B	C	D	E	F	G	H	I	J	K	L

Autumn HT+HS S3-D1	6	91.967	A	B	C	D	E	F	G	H	I	J	K	L	
Summer HS+HT S3-D1	6	91.830	A	B	C	D	E	F	G	H	I	J	K	L	
Summer HS+HT S2-D2	6	91.825	A	B	C	D	E	F	G	H	I	J	K	L	
Summer HT+HS C-D2	6	91.707	A	B	C	D	E	F	G	H	I	J	K	L	
Autumn HS+HT S3-D1	6	91.393	A	B	C	D	E	F	G	H	I	J	K	L	
Spring HT+HS C-D2	12	91.032	A	B	C	D	E	F	G	H	I	J			
Summer HS+HT S3-D2	6	90.993	A	B	C	D	E	F	G	H	I	J	K	L	
Summer HT+HS S1-D1	6	90.978	A	B	C	D	E	F	G	H	I	J	K	L	
Spring HS+HT C-D2	12	90.767	A	B	C	D	E	F	G	H	I	J			
Autumn HS+HT S1-D1	6	90.355	A	B	C	D	E	F	G	H	I	J	K	L	M
Spring HS+HT S1-D2	12	90.221		B	C	D	E	F	G	H	I	J	K		
Summer HT+HS S2-D2	6	90.137	A	B	C	D	E	F	G	H	I	J	K	L	M
Summer HT+HS S3-D1	6	89.970	A	B	C	D	E	F	G	H	I	J	K	L	M
Winter HS+HT S3-D2	12	89.402			C	D	E	F	G	H	I	J	K	L	
Summer HS+HT S2-D1	6	89.323	A	B	C	D	E	F	G	H	I	J	K	L	M

Summer HT+HS S2-D1	6	89.220	A	B	C	D	E	F	G	H	I	J	K	L	M
Autumn HS+HT S2-D1	6	88.513	A	B	C	D	E	F	G	H	I	J	K	L	M
Spring HT+HS S1-D2	12	88.464				D	E	F	G	H	I	J	K	L	
Winter HT+HS S3-D2	12	88.053					E	F	G	H	I	J	K	L	M
Summer HT+HS S3-D2	6	88.010	A	B	C	D	E	F	G	H	I	J	K	L	M
Spring HS+HT S2-D2	12	87.107						F	G	H	I	J	K	L	M
Spring HT+HS S2-D2	12	86.571						F	G	H	I	J	K	L	M
Autumn HT+HS S2-D1	6	86.537			C	D	E	F	G	H	I	J	K	L	M
Spring HT+HS S3-D2	12	85.274						F	G	H	I	J	K	L	M
Spring HS+HT S3-D2	12	84.337						F	G	H	I	J	K	L	M
Autumn HS+HT C-D2	6	80.308							G	H	I	J	K	L	M
Autumn HT+HS S2-D2	6	77.633								H	I	J	K	L	M
Autumn HT+HS C-D2	6	76.985									I	J	K	L	M
Autumn HT+HS S3-D2	6	76.945									I	J	K	L	M
Autumn HT+HS S1-D2	6	76.663										J	K	L	M

Autumn HS+HT S3-D2	6	75.137	K	L	M
Autumn HS+HT S2-D2	6	74.727		L	M
Autumn HS+HT S1-D2	6	72.795			M

Apendex B

Graphic display of the research

In this section, some of the key analyses that were undertaken in this research are demonstrated in pictorial form. The key aspects covered include samples of milk for ethanol stability test, total solid, and dry sedimentation.

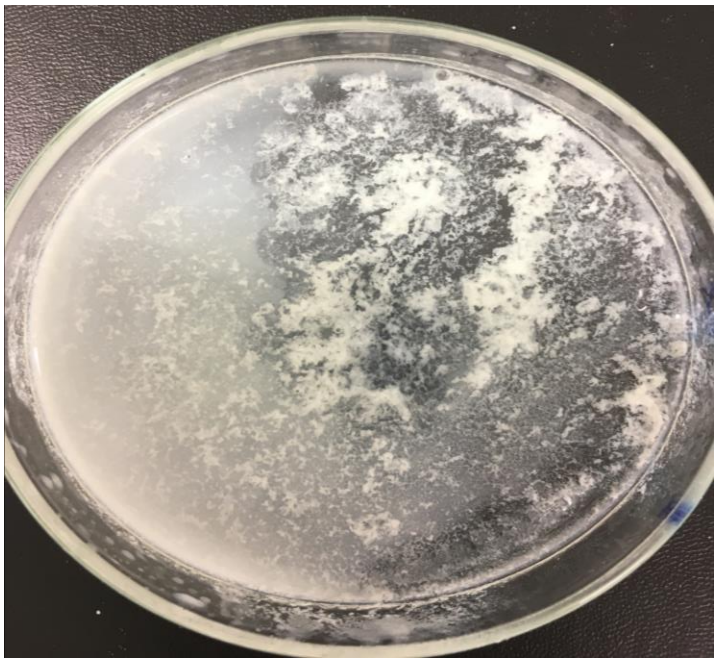

Fig. B 1: method for measuring heat stability by adding ethanol 99% to FWM.

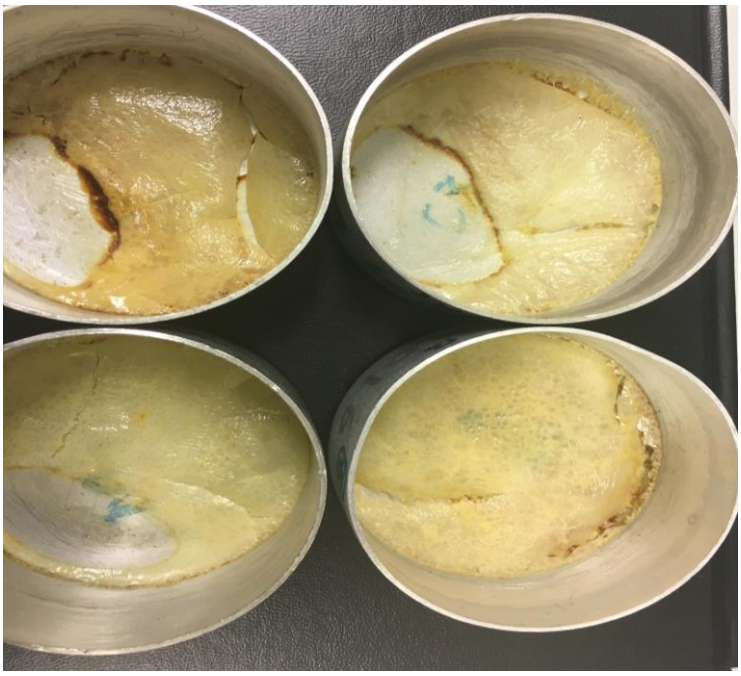

Fig. B 2: method for measuring total solid in milk FWM.

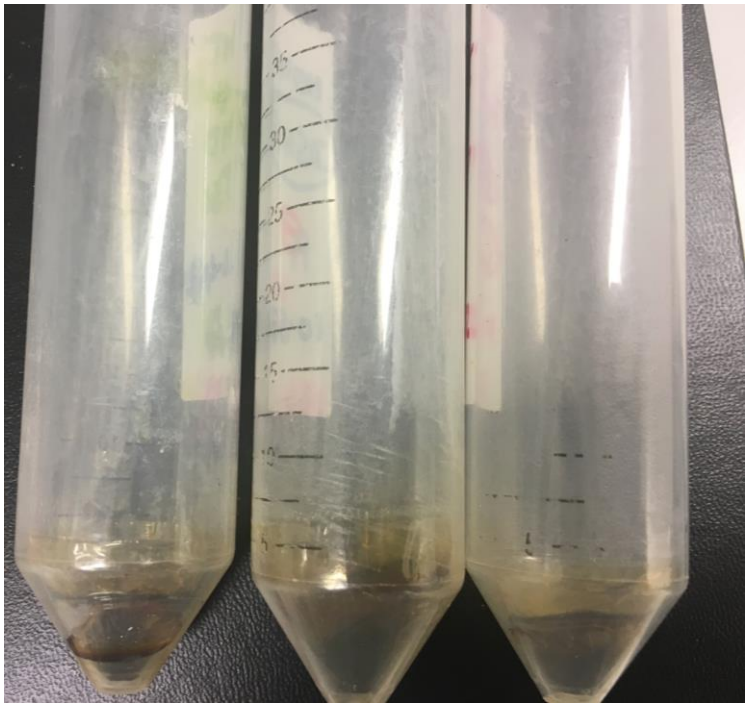

Fig. B 3: method for measuring dry sedimentation in milk FWM

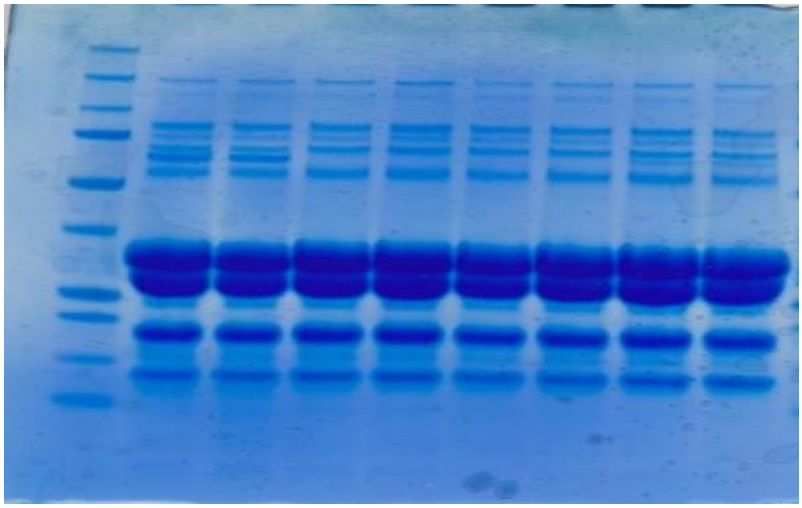

Fig. B 4: SDS image for measuring the protein composition of milk